Intracellular Accumulation of Ascorbic Acid Is Inhibited by Flavonoids via Blocking of Dehydroascorbic Acid and Ascorbic Acid Uptakes in HL-60, U937 and Jurkat Cells

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ABSTRACT In HL-60, U937 and Jurkat cells, the intracellular accumulation of ascorbic acid occurred via uptakes of both dehydroascorbic acid (an oxidized metabolite of ascorbic acid) and ascorbic acid (vitamin C). Dehydroascorbic acid and ascorbic acid were transported into cells by sodium-independent glucose transporters (GLUT 1 and GLUT 3) and sodium-dependent ascorbic acid transporters, respectively. Flavonoids inhibited the intracellular accumulation of ascorbic acid by blocking dehydroascorbic acid and ascorbic acid uptakes in the transformed cells. At flavonoid concentrations of 10–70 μmol/L, ~50% of dehydroascorbic acid uptake was inhibited in the cells. In Jurkat cells, two potent flavonoids (myricetin and quercetin) competitively inhibited dehydroascorbic acid uptake, and K_i values were ~14 and 15 μmol/L, respectively. Because GLUT 1 and GLUT 3 transport dehydroascorbic acid, the inhibition of dehydroascorbic acid uptake by flavonoids was investigated by using Chinese hamster ovary cells overexpressing rat GLUT 1 or human GLUT 3. Myricetin at concentrations of 22 and 18 μmol/L, respectively, inhibited half of dehydroascorbic acid uptake in the cells overexpressing GLUT 1 and GLUT 3. Myricetin also inhibited ascorbic acid uptake; inhibition was noncompetitive with K_i = 14 μmol/L in Jurkat cells. These data indicate that flavonoids inhibit both ascorbic acid and dehydroascorbic acid uptake but do so by different mechanisms. These data may contribute to new understanding of the biological effect of flavonoids on the intracellular accumulation of ascorbic acid in human cells. J. Nutr. 130: 1297–1302, 2000.

KEY WORDS: • *ascorbic acid* • *dehydroascorbic acid* • *U937* • *HL-60* • *and Jurkat* • *flavonoids* • *glucose transporters (GLUT 1 and GLUT 3)*

Flavonoids are a group of phytopolyphenols that have been investigated to determine their biological functions in vitro and in vivo (Harborne 1992, Macheix et al. 1990). Various biological effects on human health have been suggested (Brandi 1992, Harborne 1992, Havsteen 1983, Rice-Evans and Packer 1996). For example, flavonoids were reported to reduce LDL oxidation (Ratty and Das 1988) and quench reactive oxygen radicals in vitro (Robak and Gryglewski 1987), thereby decreasing the risk of cardiovascular diseases and cancer (Kandaswami et al. 1991, Komatus et al. 1997, So et al. 1996). These biological effects of flavonoids seem to be attributed mainly to their antioxidant properties rather than to their chemical structures. However, flavonoids were recently reported to be potential inhibitors of glucose uptake, and the structural structure was suggested to be responsible for the inhibition in monocytic U937 and lymphocytic Jurkat cells (Park 1999).

Cells use two mechanisms to accumulate ascorbate (vitamin C, ascorbic acid) (Welch et al. 1995). In one mechanism, ascorbate is transported in a sodium-dependent fashion, and two sodium-dependent ascorbate transporters were recently cloned (Tsukaguchi et al. 1999). Another mechanism is based on oxidation of external ascorbate to dehydroascorbic acid. Dehydroascorbic acid is transported into cells by sodium-independent glucose transporters GLUT 1[2] and GLUT 3 and then immediately reduced to ascorbate by intracellular proteins such as glutaredoxin (Park and Levine 1996, Rumsey et al. 1997).

Because glucose transport is inhibited by flavonoids (Park 1999) and because dehydroascorbic acid (an oxidized metabolite of ascorbic acid) is transported via some glucose transporters, we decided to investigate whether flavonoids inhibited dehydroascorbic acid transport in cells. As part of these experiments, we also investigated the effect of flavonoids on ascorbic acid transport. Interaction between flavonoids and dehydroascorbic acid was possible because the former were reported to contain antioxidant properties (Bors et al. 1995). However, the interactive effect of flavonoids on the intracellular accumulation of ascorbic acid was not studied with respect to dehydroascorbic acid transport pathway (Kuo 1998). Therefore, in this paper, the interactive effect on dehy-

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[2] Abbreviations used: CHO, Chinese hamster ovary; DTT, dithiothreitol; FBS, fetal bovine serum; GLUT, glucose transporter; PBS, phosphate buffered saline.

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droascorbic acid transport was investigated in HL-60, U937 and Jurkat cells.

MATERIALS AND METHODS

Materials. Flavonoids were purchased from Sigma (St. Louis, MO). Jurkat, U937, HL-60 and Jurkat cells were purchased from ATCC (Rockville, MD).

Cell culture conditions. U937 Jurkat and HL-60 cells were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS). Cell viability was determined microscopically by trypan blue exclusion (Grankvist et al. 1977). The cells were grown to 1–2 $\times$ 10^9/L for the uptake experiments. Cell number was counted by hematocytometer.

Dehydroascorbic acid preparation and uptake assays. [^{14}C]Dehydroascorbic acid was prepared from [^{14}C]ascorbic acid (61 Bq/mmol; NEN Life Science Products, Boston, MA) as described elsewhere (Levine et al. 1995). Briefly, 5 μL of bromine solution (Fluka, Ronkonkoma, NY) was added to 600 μL of [^{14}C] ascorbic acid at a concentration of 20 mmol/L, vortex-mixed briefly and immediately purged with nitrogen on ice in the dark for 10 min. For the uptake assay of dehydroascorbic acid, 1–2 $\times$ 10^6 cells were resuspended in 1 mL HEPES phosphate buffer containing NaCl,147 mmol/L; KCl, 5 mmol/L; KH_2PO_4, 1.9 mmol/L; Na_2HPO_4, 1.1 mmol/L; glucose, 1 mmol/L; $MgSO_4 \cdot 7H_2O$, 0.3 mmol/L; $MgCl_2 \cdot 6H_2O$, 1 mmol/L; $CaCl_2 \cdot 2H_2O$, 0.3 mmol/L; and HEPES,10 mol/L (pH 7.4). The reaction was initiated by adding [^{14}C]dehydroascorbic acid. After 30 min the reaction was terminated by washing the cells twice in cold phosphate buffered saline (PBS) (pH 7.4) (Welch et al. 1995). Uptake activity was measured in whole cells by using scintillation spectrometry or was calculated as ascorbic acid reduced from dehydroascorbic acid by using HPLC with coulometric electrochemical detection as described previously (Washko et al. 1989).

Ascorbic acid uptake assay. Ascorbic acid uptake was measured as described for dehydroascorbic acid uptake except that [^{14}C] ascorbic acid was used instead of [^{14}C] dehydroascorbic acid. For the study of ascorbic acid uptake inhibition, 1–2 $\times$ 10^6 cells were resuspended in 1 mL of the same HEPES phosphate buffer (pH 7.4) as used for dehydroascorbic acid uptake except that it contained dithiothreitol (DTT) to prevent oxidation of ascorbic acid. The various concentrations of flavonoids were added into the reaction mixtures and incubated for 2 min at room temperature. The reaction was initiated by adding [^{14}C]ascorbic acid (specific activity 61 Bq/mmol). After 4 h the reaction was terminated by washing the cells twice in cold PBS (pH 7.4). Uptake activity was measured in whole cells by using scintillation spectrometry. To test the sodium dependence of the blocking activities of flavonoids, the sodium-free buffer was prepared by replacing NaCl and Na_2HPO_4 in HEPES buffer with choline chloride and K_2HPO_4. The total mass of ascorbic acid was also calculated by using HPLC with coulometric electrochemical detection as described previously (Washko et al. 1989).

Glucose uptake assay. For this assay, 1–2 $\times$ 10^6 cells were suspended in 1 mL HEPES phosphate buffer containing NaCl,147 mmol/L; KCl, 5 mmol/L; KH_2PO_4,1.9 mmol/L; Na_2HPO_4, 1.1 mmol/L; glucose, 5.5 mmol/L; $MgSO_4 \cdot 7H_2O$, 0.3 mmol/L; $MgCl_2 \cdot 6H_2O$, 1 mmol/L; $CaCl_2 \cdot 2H_2O$, 0.3 mmol/L; and HEPES, 10 mmol/L (pH 7.4). The reaction was initiated by adding 37 Bq 2-[1,2-^{3}H(N)]deoxy-D-glucose (specific activity 969.4 Bq/mmol; NEN Life Science Products). After 10 min the reaction was terminated by washing the cells twice in cold PBS (pH 7.4) (Nishimura et al. 1993). Uptake activity was measured in whole cells by using scintillation spectrometry.

Inhibition of dehydroascorbic acid uptake by flavonoid in Chinese hamster ovary (CHO) cells overexpressing GLUT 1 or GLUT 3. CHO cells were transfected with pcDNA 3.1 expression vector (Invitrogen, Carlsbad, CA) containing rat GLUT 1 or human GLUT 3 (Chen and Okayama 1987, Inukai et al. 1995, Rumsey et al. 1997). After the transfection, the cells were maintained in Ham's F-12 with 10% fetal calf serum and antibiotic G418 (600 mg/L) (Rumsey et al. 1997). To measure the inhibition of dehydroascorbic acid uptake in CHO cells, confluent cells in six-well plates were twice washed with Krebs buffer (HEPES, 30 mmol/L; NaCl, 130 mmol/L; KH_2PO_4, 4 mmol/L; $MgSO_4$, 1 mmol/L; $CaCl_2$, 1 mmol/L; pH 7.4) and incubated at room temperatures for 5 min with Krebs buffer containing different concentrations of flavonoids. As described above, the reaction was initiated by adding [^{14}C]dehydroascorbic acid. After 30 min the reaction was terminated by washing twice the cells in cold PBS (pH 7.4), suspended in PBS containing NaOH (0.1 mol/L) and CHAPS (10 g/L; Calbiochem-Nvabiochem, La Jolla, CA), and radioactivity was measured by scintillation spectrometry.

Kinetic analyses. Analyses of the inhibition of the dehydroascorbic acid and ascorbic acid uptakes by flavonoids were performed (Neale and Richards 1972), and the values of K_i were determined by using a Lineweaver-Burk plot. Data points in all figures represent the means of three or more samples $\pm$ SD.

RESULTS

Inhibition of intracellular accumulation of ascorbic acid. Several flavonoids and ascorbic acid were coincubated in cell cultures to determine whether flavonoids inhibit the intracellular accumulation of ascorbic acid. Flavonoids inhibited intracellular accumulation of ascorbic acid (**Table 1**). Flavonoid concentrations necessary to inhibit 50% of ascorbic acid accumulation were 20–80 μmol/L in Jurkat, U937 and HL-60 cells. The inhibitory effect of flavonoids was also investigated in adherent HeLa cells, and the concentrations required to inhibit 50% of ascorbic acid accumulation were slightly higher, ~100 μmol/L (data not shown). These differences might be attributed to different expression patterns of the transporters involved in the accumulation of ascorbic acid or the difference of growth modes of these cell lines. Surprisingly, the tea flavonoid catechin was not inhibitory even at 500 μmol/L (data not shown).

Inhibition of dehydroascorbic acid uptake by flavonoids. Because ascorbic acid can oxidize to dehydroascorbic acid during experiments, the effects of flavonoids on ascorbic acid accumulation (Table 1) could be due to inhibition of ascorbic acid uptake, inhibition of dehydroascorbic acid uptake or both. To distinguish among these possibilities, flavonoids were investigated for their inhibitory effect on dehydroascorbic acid uptake and were found to inhibit dehydroascorbic acid uptake in Jurkat cells; inhibition of uptake by 50% occurred between 10 and 70 μmol/L (**Fig. 1**). Flavonoids inhibited dehydroascorbic acid uptake independent of sodium in the buffer (data not shown), indicating that the inhibition of dehydroascorbic acid uptake occurs via blocking the sodium-independent transporters. Because myricetin and quercetin were

TABLE 1

Concentrations of flavonoids that inhibit half of intracellular accumulation of ascorbic acid in HL-60, U937 and Jurkat cells[1,2]

Flavonoid	Jurkat	U937	HL-60
		μmol/L	
Myricetin	20 $\pm$ 3	25 $\pm$ 5	20 $\pm$ 4
Quercetin	25 $\pm$ 4	27 $\pm$ 5	24 $\pm$ 3
Fisetin	30 $\pm$ 5	34 $\pm$ 4	35 $\pm$ 3
Apigenin	40 $\pm$ 4	45 $\pm$ 4	46 $\pm$ 7
Naringenin	60 $\pm$ 5	65 $\pm$ 6	63 $\pm$ 3
Hesperetin	75 $\pm$ 5	70 $\pm$ 7	80 $\pm$ 5

[1] Values represent the means $\pm$ SD, $n > 3$.

[2] The cells were cultured for 4 h in RPMI with 10% fetal bovine serum in the presence of ascorbic acid (100 μmol/L) and flavonoids (0, 15, 30, 45, 60, 75, 80 and 100 μmol/L).

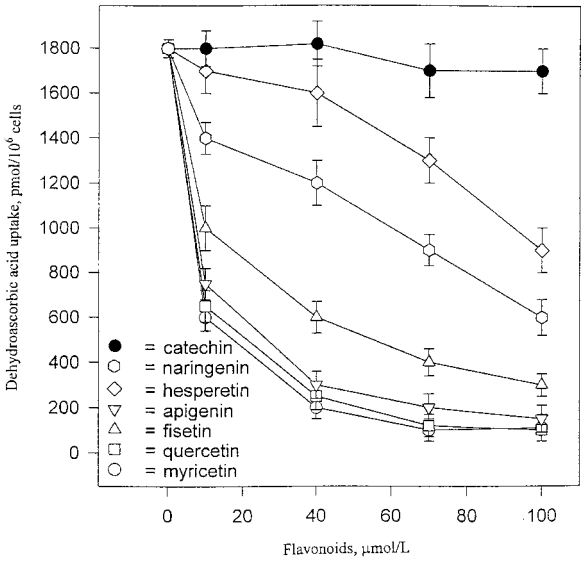

FIGURE 1 Inhibition of dehydroascorbic acid uptake in Jurkat cells by flavonoids. Dehydroascorbic acid uptake (150 μmol/L) was inhibited by flavonoids: myricetin, quercetin, fisetin, apigenin, hesperetin, naringenin and catechin. Data points represent the mean ± SD, $n \geq 3$.

effective inhibitors of dehydroascorbic acid uptake in monocytic (HL-60 and U937) and lymphocytic (Jurkat) cells, kinetic studies were performed using these two flavonoids in Jurkat cells. Dehydroascorbic acid uptake was competitively inhibited by myricetin and quercetin, and K_i values for these flavonoids were 14 and 15 μmol/L, respectively (**Fig. 2**). K_i values for myricetin determined in HL-60 and U937 cells were ~15 and 14 μmol/L, respectively, similar to values in Jurkat cells. These data show that flavonoids were competitive inhibitors of dehydroascorbic acid uptake in a sodium-independent manner.

Inhibition of dehydroascorbic acid uptake in CHO cells overexpressing GLUT 1 or GLUT 3. Dehydroascorbic acid uptake occurs via GLUT1 and GLUT3, and the inhibitory effect of flavonoids on these two transporters might be different, necessitating different concentrations for inhibition of dehydroascorbic acid uptake in the different cells. To address this issue and to confirm that flavonoids inhibit dehydroascorbic acid uptake by blocking GLUT 1 and GLUT 3, dehydroascorbic acid uptake was compared in wild-type CHO cells and CHO cells overexpressing GLUT 1 (GLUT 1:CHO). GLUT 1:CHO cells transported four to five times more dehydroascorbic acid than did the wild-type CHO cells (**Fig. 3**), demonstrating that GLUT 1 is involved in dehydroascorbic acid uptake. Dehydroascorbic acid uptake in GLUT 1:CHO cells was inhibited by myricetin (Fig. 3), indicating that the flavonoid inhibits GLUT 1 (IC_{50} ~22 μmol/L). The same experimental design was used to test CHO cells overexpressing GLUT 3 (GLUT 3:CHO). As seen in GLUT 1:CHO cells, dehydroascorbic acid uptake was three to five times greater in the GLUT 3:CHO cells than in wild-type CHO cells (Fig. 3). Myricetin inhibited the increased dehydroascorbic acid uptake in the GLUT 3:CHO cells (IC_{50} ~ 18 μmol/L). These data suggested that myricetin effectively inhibited dehydroascorbic acid uptake in GLUT 3:CHO cells at slightly lower concentrations than in GLUT 1:CHO cells. The heterogeneity of these two glucose transporters might explain why different concentrations of flavonoids were required to inhibit dehydroascorbic acid uptake in the suspension cells and the adherent cells. If flavonoids inhibited dehydroascorbic acid uptake by blocking GLUT 1 and GLUT 3 as demonstrated above, flavonoids should inhibit glucose uptake, to some extent, in the tumor cells used in this study. Flavonoids inhibited glucose uptake in HL-60, U937 and Jurkat cells ($K_i \geq 10$ μmol/L; data not shown). The data indicate that flavonoids blocked glucose transporters GLUT 1 and GLUT 3, thus inhibiting dehydroascorbic acid as well as glucose uptakes in the cells.

Inhibition of ascorbic acid uptake by flavonoids. Whether ascorbic acid exists as such or in an oxidized form (dehydroascorbic acid) depends on the redox conditions of cell culture. Thus it is possible that the results in Table 1 could be explained by oxidation of ascorbic acid to dehydroascorbic acid and inhibition of dehydroascorbic acid uptake by flavonoids. The results could also be explained by flavonoid inhibition of ascorbic acid uptake distinct from the effects on dehydroascorbic acid uptake. We performed experiments to discriminate between the two possibilities and examine whether ascorbic acid uptake is inhibited by flavonoids. Experiments investigating ascorbic acid transport required longer incubation times than did those investigating dehydroascorbic acid. Because extended incubation might result in the further oxidation of ascorbic acid into dehydroascorbic acid, we added DTT at 0.1 mmol/L to the reaction mixtures. DTT at 0.1 mmol/L maintains ascorbic acid in reduced form for > 4 h. In contrast to dehydroascorbic acid uptake, ascorbic acid uptake is sodium-dependent (**Fig. 4**A). This characteristic sodium dependency of ascorbic acid uptake was not altered by the presence of DTT (data not shown).

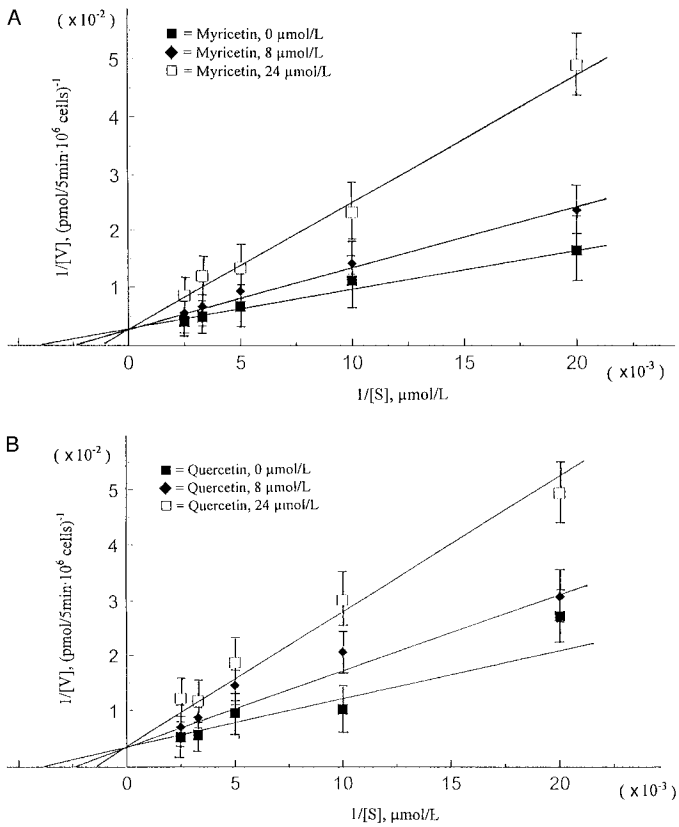

FIGURE 2 Competitive inhibition of dehydroascorbic acid uptake in Jurkat cells by myricetin and quercetin. The values of K_i for myricetin (*A*) and quercetin (*B*) were determined using Lineweaver-Burk plot. Transports of dehydroascorbic acid at 50, 100, 200, 300 and 400 μmol/L were measured in the presence of 0, 8 and 24 μmol/L of myricetin or quercetin. Data points represent the means ± SD, $n \geq 3$.

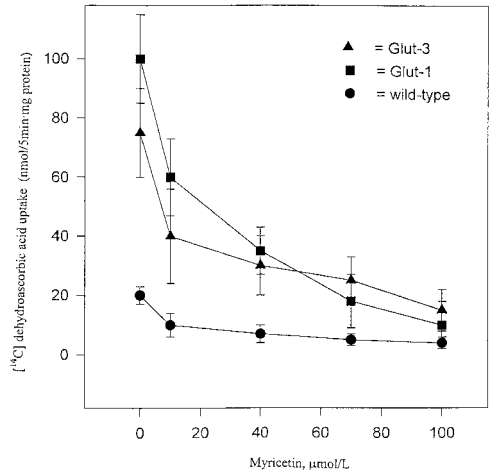

FIGURE 3 Inhibition of dehydroascorbic acid uptake in Chinese hamster ovary (CHO) cells overexpressing glucose transporter GLUT-1 or GLUT-3. Wild-type CHO cells and CHO cells overexpressing GLUT-1 and GLUT-3 were incubated in six-well plates at room temperature for 5 min in Krebs medium containing the indicated concentrations of flavonoid and 150 μmol/L [^{14}C]dehydroascorbic acid. Cells were then washed three times with ice-cold phosphate-buffer saline (PBS), and lysed in 0.5 mL PBS containing NaOH (0.1 mol/L) and CHAPS (10 g/L). Cell-associated radioactivity was measured with scintillation spectrometry. Data points represent the means ± SD, $n > 3$.

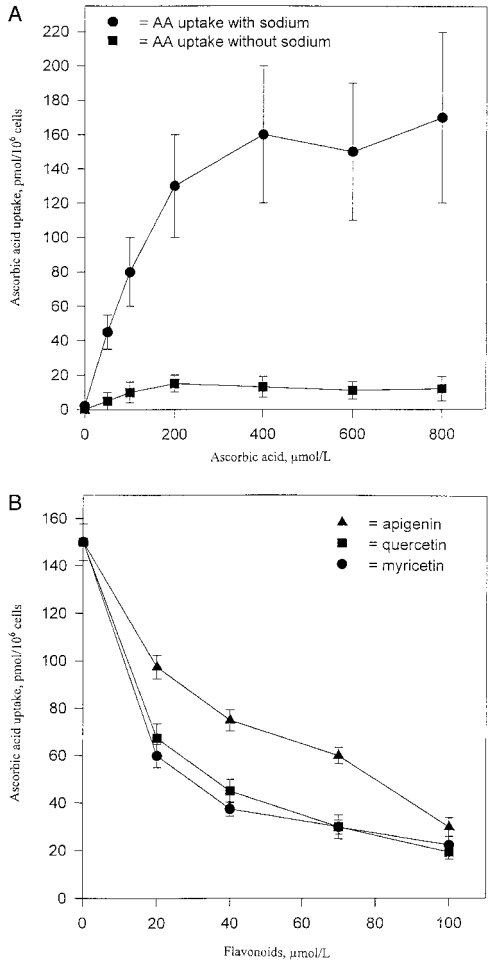

FIGURE 4 Ascorbic acid (AA) uptake and its inhibition by flavonoids in Jurkat cells. *A*) Ascorbic acid uptake was measured at the concentrations ranged from 0 to 800 μmol/L. in the presence or absence of sodium. *B*) Ascorbic acid uptake occurring at 100 μmol/L was inhibited by myricetin, quercetin and apigenin at the indicated concentrations. Data points represent the mean ± SD, $n > 3$.

To confirm that flavonoids inhibited ascorbic acid uptake specifically and to study the mechanism of inhibition, Jurkat cells were incubated with flavonoids and ascorbic acid in the presence of DTT. All three flavonoids (myricetin, quercetin and apigenin) inhibited ascorbic acid uptake (IC_{50} values for myricetin, quercetin and apigenin were ~16, 17 and 40 μmol/L, respectively; Fig. 4*B*). Similar findings were observed in U937 and HL-60 cells (data not shown). These data show that flavonoids inhibited not only sodium-independent dehydroascorbic acid uptake, but also sodium-dependent ascorbic acid uptake. In Jurkat cells, myricetin inhibited sodium-dependent ascorbic acid uptake noncompetitively with a K_i of 14 μmol/L (**Fig. 5**). Quercetin and apigenin also inhibited ascorbic uptake noncompetitively (data not shown).

Flavonoid-reducing activity and inhibition of dehydroascorbic acid uptake. Because dehydroascorbic acid uptake is faster than ascorbic acid uptake and flavonoids were reported to have antioxidant properties, it was possible that flavonoid inhibition of ascorbic acid accumulation (Table 1) could also be due to flavonoid reduction of dehydroascorbic acid to ascorbic acid. Therefore, we investigated the potential effect of the antioxidant properties of flavonoids on dehydroascorbic acid uptake. First, we determined whether flavonoids reduced dehydroascorbic acid to ascorbic acid under the experimental conditions described here. Using HPLC with an electrochemical detector, we did not detect reduction by flavonoids even at myricetin and quercetin concentrations as high as 200 μmol/L (data not shown). To further explore the inhibitory effect of flavonoids, we used flavonoids with structural modifications that would affect their antioxidant properties; 5,7,4′-hydroxy flavone (apigenin) inhibited both dehydroascorbic acid and glucose uptake in Jurkat cells. If these inhibitions were due to the antioxidant property of 5,7,4′-hydroxy flavone, 5,7,4′-trimethoxy flavone could not inhibit dehydroascorbic acid and glucose uptake in the cells. We found that 5,7,4′-trimethoxy flavone inhibited dehydroascorbic acid as well as glucose uptakes in Jurkat cells (data not shown). The same conclusion was obtained using 3,5,7,3′,4′-pentamethoxy flavone (data not shown). Taken together, these experiments indicated clearly that the inhibition of dehydroascorbic acid uptake results from the structural char-

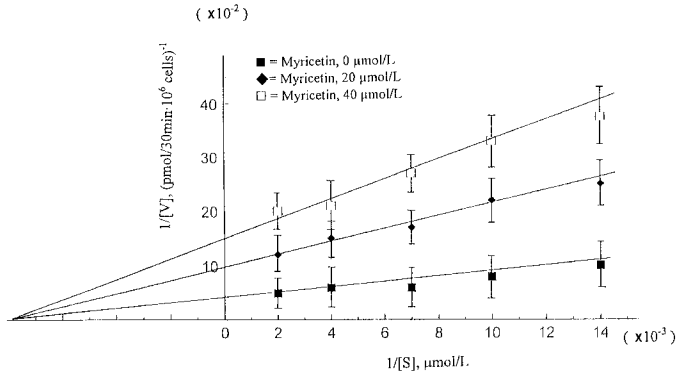

FIGURE 5 Noncompetitive inhibition of ascorbic acid uptake in Jurkat cells by myricetin. The value of K_i for myricetin was determined by using a Lineweaver-Burk plot. Transport of ascorbic acid at 70, 100, 140, 250 and 500 μmol/L was measured in the presence of 0, 20 and 40 μmol/L of myricetin. Data points represent the mean ± SD, $n > 3$.

acteristics of flavonoids and not from their antioxidant properties.

DISCUSSION

The interaction between flavonoids and nutrients is important to human nutrition because flavonoids are usually consumed with other nutrients found in foods (Brandi 1992, Gee et al.1998, Havsteen 1983, Hertog and Hollman 1996, Macheix et al. 1990, Noteborn et al. 1997). However, the past studies of flavonoids have not considered possible interactions between flavonoids and nutrients, thus not considering their collateral effect on each other. In particular, the effect of flavonoids on the uptake of ascorbic acid has not been investigated in detail even though interactions between flavonoids and ascorbic acid have been suspected (Bors et al. 1990 and 1995). Because flavonoids were reported to inhibit glucose uptake in cells and because dehydroascorbic acid uptake occurs via sodium-independent glucose transporters, we investigated the inhibitory effect of flavonoids on the intracellular accumulation of ascorbic acid. In HL-60, U937 and Jurkat cells, the simultaneous incubation of ascorbic acid and flavonoids yielded a large decrease in intracellular accumulation of ascorbic acid. The most effective flavonoids were quercetin, myricetin and fisetin, which belong to the flavonol group. On the basis of the heterocyclic C-ring of flavonoids and the interlinkage between benzopyran and benzene groups, flavonoids are classified into several subgroups: the flavan, flavone, flavonol, flavonone, isoflavone and anthocyanin groups (Park 1999). Since flavonoids in the flavonol group were most effective in inhibiting both dehydroascorbic acid and glucose uptake in HL-60, U937 and Jurkat cells, the chemical structure of flavonoids seems important in blocking the cellular uptakes of dehydroascorbic acid and glucose. Previously, the structural effect had been demonstrated by investigating the inhibitory activities of flavonoid analogs on glucose uptake in the cells (Park 1999). For verification, a similar experiment was performed on dehydroascorbic acid uptake, and similar data were obtained (data not shown). Therefore, the effects of flavonoids on dehydroascorbic acid uptake were shown to be due to their structural properties.

Intracellular accumulation of ascorbic acid seems to occur via two separate transport pathways: dehydroascorbic acid transport and ascorbic acid transport (Welch et al. 1995). Ascorbic acid might be oxidized to dehydroascorbic acid under cell culture conditions. Dehydroascorbic acid uptake was reported to take place via sodium-independent transporters (GLUT 1 and GLUT 3), and the transported dehydroascorbic acid was reduced immediately to ascorbic acid in the cells (Park and Levine 1996, Rumsey et al. 1997). These findings were reconfirmed here. Dehydroascorbic acid uptake occurred in the cells (Jurkat, HL-60, U937 and HeLa) independent of sodium in the buffer. This uptake was measured by using both ^{14}C-labeled dehydroascorbic acid and HPLC to calculate actual mass of ascorbic acid reduced from dehydroascorbic acid. We also demonstrated, by using CHO cells overexpressing GLUT 1 or GLUT 3, that dehydroascorbic acid uptake occurred via these two glucose transporters and that flavonoids inhibited dehydroascorbic acid uptake by blocking these transporters.

If flavonoids inhibit the intracellular accumulation of ascorbic acid by blocking the glucose transporters (GLUT 1 and GLUT 3), flavonoids should inhibit glucose uptake to some extent, especially via GLUT 1 and GLUT 3. This proposition was verified by data presented here and previously (Park 1999, Vera et al. 1996). Flavonoids simultaneously inhibited dehydroascorbic acid and glucose uptakes in HL-60, U937 and Jurkat cells. In HeLa cells, flavonols also inhibited the dehydroascorbic acid uptake; the concentration required to inhibit 50% of dehydroascorbic acid uptake was ~20–100 μmol/L, similar to results for Jurkat, U937 and HL-60 cells. However, glucose uptake of HeLa cells was different from those of HL-60, U937 and Jurkat cells. In HeLa cells, glucose uptake seems to result not only from sodium-independent transport, but also from sodium-dependent transport (Data not shown). Therefore, at the concentration of 20 μmol/L, flavonols could not inhibit glucose uptake of the cells, due to sodium-dependent glucose uptake. The data indicate clearly that flavonoids block sodium-independent glucose transporters, thus inhibiting dehydroascorbic acid uptake.

Ascorbic acid is also transported into cells via a sodium-dependent pathway. Under the normal conditions of cell culture and in vitro experiments, ascorbic acid is partially oxidized to dehydroascorbic acid. The oxidation of ascorbic acid enables the uptake of both residual ascorbic acid and converted dehydroascorbic acid. To prevent this bilateral uptake, ascorbic acid uptake was studied in the presence of DTT, an artificial reductant, which prevented ascorbic acid from being oxidized to dehydroascorbic acid for >4 h. The addition of DTT did not change sodium-dependent ascorbic acid uptake. However, a longer incubation (at least 3 h) is required for reasonable scintillation spectrometry and HPLC measurement because sodium-dependent ascorbic acid uptake is slower than sodium-independent dehydroascorbic acid uptake. In some studies this extended incubation was commonly implemented to measure ascorbic acid uptake (Kuo 1998, Rumsey et al. 1997, Welch et al. 1995). As described previously, myricetin noncompetitively inhibited sodium-dependent ascorbic acid uptake under these conditions.

Recently, cDNA for a sodium-dependent transporter for ascorbic acid (rSVCT1 and rSVCT2) were isolated from rat kidney and characterized extensively (Tsukaguchi et al. 1999); phloretin, a flavonoid-like molecule, inhibited ascorbic acid uptake in oocytes expressing rSVCT1. Our data are consistent with this observation in rSVCT1. Human counterpart cDNA (hSVCT1 and hSVCT2) for rat ascorbic acid transporters (rSVCT1 and rSVCT2) were also reported (Faaland et al. 1998), but they were not thoroughly investigated to ascertain whether these clones had transport activity for ascorbic acid. Because our study was performed with transformed human cells to determine the effect of flavonoids on activity of ascorbic acid uptakes, a series of experiments are currently underway using human ascorbate transporters that are expressed in *Xenpous laevis* or overexpressed in cells. Further investigation might elucidate how flavonoids inhibit sodium-dependent ascorbic acid transporters and what moiety of flavonoids is required to inhibit ascorbic acid uptake. Specific inhibitors for ascorbic acid uptake have been sought by designing ascorbic acid analogs in order to characterize its transporters. In this paper, flavonoids were suggested as natural inhibitors for dehydroascorbic acid and ascorbic acid uptake in vitro. Because the average human intake of flavonoids was recently estimated to be ~23 mg/d (Hertog et al. 1993), the effect of flavonoids on the absorption of ascorbic acid has to be investigated in the intestine to determine true bioavailability of ascorbic acid. Flavonoids might be used as a valuable tool to characterize absorption and transporters of ascorbic acid in the future because hundreds of different flavonoids are available in nature.

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