Transport of Cranberry A-Type Procyanidin Dimers, Trimers, and Tetramers across Monolayers of Human Intestinal Epithelial Caco-2 Cells

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ABSTRACT: A-type procyanidin oligomers in cranberries are known to inhibit the adhesion of uropathogenic bacteria. B-type procyanidin dimers and trimers are absorbed by humans. The absorption of A-type procyanidins from cranberries in humans has not been demonstrated. This study examined the transport of A-type cranberry procyanidin dimers, trimers, and tetramers on differentiated human intestinal epithelial Caco-2 cell monolayers. Procyanidins were extracted from cranberries and purified using chromatographic methods. Fraction I contained predominantly A-type procyanidin dimer A2 [epicatechin-(2-O-7, 4−8)-epicatechin]. Fraction II contained primarily A-type trimers and tetramers, with B-type trimers, A-type pentamers, and A-type hexamers being minor components. Fraction I or II in solution was added onto the apical side of the Caco-2 cell membranes. The media at the basolateral side of the membranes were analyzed using HPLC−MS^n after 2 h. Data indicated that procyanidin dimer A2 in fraction I and A-type trimers and tetramers in fraction II traversed across Caco-2 cell monolayers with transport ratio of 0.6%, 0.4%, and 0.2%, respectively. This study demonstrated that A-type dimers, trimers, and tetramers were transported across Caco-2 cells at low rates, suggesting that they could be absorbed by humans after cranberry consumption.

KEYWORDS: *A-type procyanidins, transport, cranberries, Caco-2 cells*

INTRODUCTION

Cranberries have been shown to be effective in preventing urinary tract infections,[1,2] dental caries,[3,4] cardiovascular diseases,[5] cancers,[6,7] and aging-related disorders.[8] These physiological functions are attributed in part to the procyanidins in cranberries. Procyanidins are oligomers and polymers that consist of (+)-catechin or (−)-epicatechin as constituent units. The most ubiquitous procyanidins in foods are the B-type procyanidins, in which the (epi)catechin units are linked through the C4→C8 or C4→C6 interflavan bonds. A-type procyanidins are less common in nature. The (epi)catechin units in A-type procyanidins are linked by an additional ether bond between C2 and O7. Over 50% of the procyanidins in cranberries are A-type.[9] A-type procyanidin oligomers isolated from cranberries were able to inhibit adherence of uropathogenic *Escherichia coli*, whereas the B-type procyanidins were not.[10,11] These previous studies suggest that A-type procyanidins are the major bioactive components in cranberries.

B-type procyanidin dimers and trimers were absorbed and present in the blood of human subjects.[12−14] An in situ intestinal perfusion experiment in anesthetized rats revealed that A-type procyanidin dimer A1 [epicatechin-(2-O-7, 4−8)-catechin] and A2 [epicatechin-(2-O-7, 4−8)-epicatechin] isolated from peanut skin were absorbed and their transport ratios were higher than that of dimer B2 [epicatechin-(4−8)-epicatechin].[14] However, A-type procyanidins were not known to be absorbed by humans. As the first step to elucidate the bioavailability of A-type cranberry procyanidins in humans, we employed human intestinal epithelial Caco-2 cells to investigate the transport of A-type procyanidins.

MATERIALS AND METHODS

Chemicals and Materials. Freeze-dried cranberries were provided by Ocean Spray Cranberries, Inc. (Lakeville−Middleboro, MA). A-type procyanidin dimer A2 (epicatechin-(2-O-7, 4−8)-epicatechin) was purchased from Chromadex Inc. (Irvine, CA). B-type procyanidin dimer [epicatechin-(4−8)-epicatechin] was obtained from Indofine Chemical Company, Inc. (Hillsborough, NJ). A B-type procyanidin standard that contained monomers to decamers was kindly provided by Mars Botanicals (Rockville, MD). Amberlite FPX 66 resin was a product from Dow Company (Midland, Michigan). Caco-2 cells originating from human colorectal carcinoma were obtained from the American Type Culture Collection (Manassas, VA). Solid-phase extraction cartridges and other reagents were obtained from Fisher Scientific Co. (Pittsburgh, PA).

Purification of Cranberry Procyanidins. Five hundred grams of spray-dried cranberry powder was extracted with 4 L of methanol at room temperature for 48 h. Extracts obtained after vacuum filtration were combined and concentrated under a partial vacuum using a rotary evaporator. The concentrated extract was resuspended in 100 mL of water and loaded onto a column (3.8 × 44 cm) packed with Amberlite FPX 66 resins. The column was eluted with 5 L of deionized water to remove sugars followed by 2 L of methanol to yield cranberry phytochemical powder (about 28 g). Part of this powder (27 g) was suspended in 80 mL of DI water and loaded onto a column (28 cm, 5.8 cm i.d.) packed with Sephadex LH-20, which was soaked in 30% methanol for over 4 h before use. The column was eluted with 30% methanol (1.6 L), 60% methanol (1.2 L), 80% methanol (1.2 L), 100% methanol (1.2 L) and 70% acetone (1.2 L). Every 400 mL eluent was collected as

a fraction. The third fraction of 80% methanol eluent was referred to as fraction I (about 1.3 g). The 100% methanol eluent was concentrated to yield 3 g of dry extract. It was suspended in 20 mL of water and partitioned with 100 mL of ethyl acetate three times. The ethyl acetate phases were combined and evaporated to yield 0.45 g of extract. This was referred to as fraction II.

Cell Culture. Caco-2 cells between passages 28 and 32 were used in these experiments. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with glucose (4.5 g/L), L-glutamine, and 20% fetal bovine serum as previously described.[15] At 80−90% confluence, cells were seeded at a level of 1×10^5 cells/cm^2 in 6-well Transwell plates (Corning Inc., Corning, NY) and cultured in an incubator at 37 °C with 5% CO$_2$. The media were changed every 2 days for the first 7 days. After 8 days, the medium in the apical chamber was changed every day while the basolateral chamber medium was changed every other day.

Transport Experiments. The transport experiment was conducted when the transepithelial electrical resistance of the Caco-2 cell membranes was >500 Ω cm^2, typically at 17 days postseeding. Briefly, growth medium was removed from the Transwells by aspiration, and the upper and lower chambers were rinsed with Hank's balanced salt solution (HBSS, pH 7.2−7.4). Then 1.5 and 2.6 mL of HBSS (pH 7.2−7.4) were added into the apical and basolateral chambers, respectively. After 30 min of preincubation the HBSS was aspirated. Fraction I or procyanidin dimer A2 standard was dissolved in 1.5 mL of HBSS (containing 0.5% DMSO) to a concentration of 1 mM A-type procyanidin dimer. A solution of fraction II with 0.4 mM A-type procyanidin trimers and 0.3 mM tetramers was used to mimic the expected concentration of procyanidins occurring in the gut.[12] These solutions were added to the apical chambers. Meanwhile, 2.6 mL of HBSS with 0.5% DMSO was added to the basolateral chambers. Transport was carried out at 37 °C for 2 h as previously described.[16] At the end of uptake the 2.6 mL solutions in basolateral chambers and the 1.5 mL solutions in apical chambers were collected. Then, each of the apical or basolateral chambers was washed with 1.0 or 2.4 mL of phosphate-buffered saline (pH 7.4). Rinsing solutions were added into the HBSS. Transport experiments were carried out on 3 individual wells for each sample. Wells with a transmonolayer electrical resistance value below 300 Ω cm^2 at the end of transport were discarded to ensure the integrity of monolayers.[17]

Solid-Phase Extraction. Procyanidins were extracted from the HBSS using C18 solid-phase extraction cartridges immediately after the transport experiments. The cartridges were washed with 3 mL of methanol and equilibrated with 3 mL of water. After HBSS solutions from apical or basolateral chambers were loaded, the cartridges were washed with 3 mL of water. Procyanidins were recovered with 3 mL of 100% methanol. The methanol eluent was dried in a SpeedVac concentrator (Fisher Scientific, Pittsburgh, PA). Dried samples were dissolved in methanol for HPLC−MS^n analyses.

HPLC−MS^n Analysis. Chromatographic analyses were performed on an Agilent 1200 HPLC system (Palo Alto, CA) equipped with a binary pump, an autosampler, a fluorescence detector, and a HCT ion trap mass spectrometer (Bruker Daltonics, Billerica, MA). Separation of procyanidins was carried out on a 250 mm × 4.6 mm i.d., 5 μm, Develosil Diol 100 Å column, with a 4 mm × 3 mm i.d. guard column (Phenomenex, Torrance, CA) at a column temperature of 35 °C. The binary mobile phase consisted of (A) acetonitrile/acetic acid (98:2, v/v) and (B) methanol/water/acetic acid (95:3:2, v/v/v). The 76 min gradient was as follows: 0−12 min, 7% B isocratic; 12−60 min, 7−37.6% B linear; 60−63 min, 37.6−100% B linear, 63−70 min 100% B isocratic; 70−76 min 100−7% B linear; followed by 5 min of re-equilibration of the column before the next run. Excitation and emission of the fluorescent detector were set at 231 and 320 nm, respectively. Electrospray ionization at negative mode was performed using nebulizer 50 psi, drying gas 10 L/min, drying temperature 350 °C, and capillary 4000 V. Mass spectra were recorded from m/z 150 to 2000. The most abundant ion in full scan was isolated, and its product ion spectra were recorded.

Determination of Procyanidin Contents, Apparent Permeability Coefficient and Transport Ratio. (−)-Epicatechin, B-type and A-type procyanidin dimers were quantitated using external standards. A-type procyanidin trimers and tetramers were quantified using B-type procyanidin standard from Mars Botanicals (Rockville, MD). Apparent permeability coefficients (P_{app}, cm/s), transport ratio and recovery rates were calculated using the following equations:

$$P_{app} = (dQ/dt) \times (1/AC_0)$$

where dQ/dt is the permeability rate (μg/s), C_0 is the initial concentration in the donor chamber (μg/mL), and A is the surface area of filter (cm^2), which is 4.67 cm^2 in this study;

$$\text{transport ratio} = (\text{procyanidin transported})$$
$$/(\text{total procyanidins}) \times 100\%$$

$$\text{recovery rate} = [\text{procyanidin (transported + remaining)}]$$
$$/(\text{total procyanidin}) \times 100\%$$

"Procyanidin transported" represents the amount of procyanidins on the basolateral side of the Transwell after transport. Total procyanidins was the total amount of procyanidins added on the apical side of the Transwell at the beginning of the experiment. Procyanidins (transported + remaining) were the sum of procyanidins that traversed across Caco-2 membranes and those that remained in the apical side after transport.

Statistical Analysis. All data were expressed as the mean ± standard deviation. One-way analyses of variance (ANOVA) with Tukey−Kramer HSD pairwise comparison of the means were performed using JMP software (Version 9.0, SAS Institute Inc., Cary, NC). A difference of $p \leq 0.05$ was considered to be significant.

■ RESULTS AND DISCUSSION

Composition of Procyanidin Fractions Purified from Cranberries. Cranberry methanol extracts contained sugars and phytochemicals such as anthocyanins, flavonols, and oligomeric and polymeric procyanidins.[18−20] Amberlite resin FPX66 was used to remove sugars from the cranberry extracts. A Sephadex LH-20 column was employed to separate anthocyanins and flavanols from procyanidins. Anthocyanins and flavanols bind to Sephadex LH-20 with lower affinity and, thus, were eluted earlier. Procyanidin oligomers and polymers bind to Sephadex LH-20 with stronger affinities and were eluted by methanol or acetone.[21] Two fractions were obtained from a Sephadex LH-20 column, and their HPLC chromatograms are illustrated in Figure 1.

The chromatogram of fraction I showed a major peak (2A) at 8.2 min and a minor peak at 17.4−18.2 min. Peak 2A was identified as procyanidin A2 because it had the same retention time and mass spectra as procyanidin A2 standard. This peak showed $[M − H]^-$ m/z 575. Its product ion at m/z 285 was due to quinone methide (QM) cleavage of A-type interflavan bonds. Product ion at m/z 423 resulted from the retro-Diels−Alder (RDA) fission of the heterocyclic ring (Table 1). The minor peaks with retention time of 17.4−18.2 min were identified as A-type procyanidin trimers according to $[M − H]^-$ m/z 863. The diagnostic ion at m/z 575 derived from QM cleavage of the interflavan bond indicated that these trimers had a connection sequence of (epi)cat−(epi)cat−A−(epi)cat. The (epi)cat denotes catechin or epicatechin since they could not be distinguished by mass spectrometer. −A− represents an A-type linkage. The A-type interflavan linkage in the trimer was between the middle unit and the terminal units. A product ion of m/z 711 was due to retro-Diels−Alder (RDA) fission. Ion m/z 287 derived from QM cleavage of B-type interflavan linkage between the up and middle units. Ion m/z 693 was generated from RDA cleavage and a loss of water (Table 1).
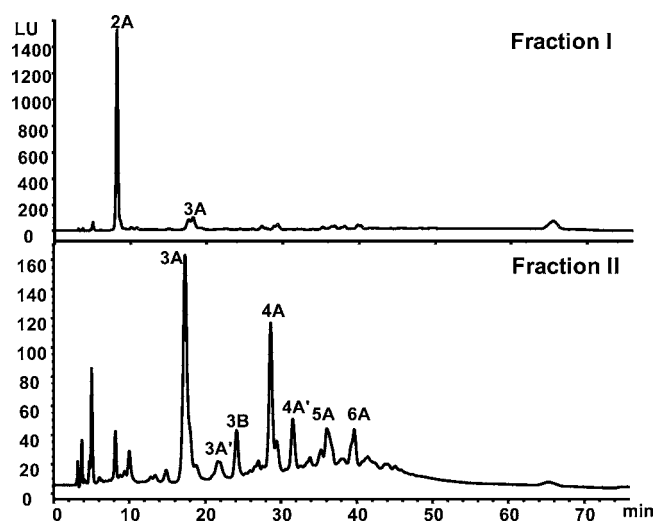
Figure 1. HPLC chromatogram of procyanidins in fraction I and fraction II. Peak 2A, 3A, 4A, 5A, and 6A were A -type dimers, trimers, tetramers, pentamers, and hexamers, respectively. Peak 3B was B-type trimers.

The content of procyanidin dimers and trimers in fraction I was 44.1% and 7.6% (w/w), respectively.

Fraction II contained oligomers with degree of polymerization between 3 and 6. Peak 3A with retention time of 17.4 min showed $[M - H]^-$ m/z 863 and product ions similar to peak 3A in fraction I. They were deduced to be the same compound. Peak 3A' at 21.6 min had a $[M - H]^-$ m/z 863 and similar product ion spectra to that of peak 3A. This peak was also an A-type trimer with connection sequence of (epi)cat—(epi)cat—A—(epi)cat. Foo et al.[11] isolated two A-type procyanidin trimers from cranberries with this same connection sequence. They were epicatechin—(4—6)-epicatechin—(4—8, 2-O-7)—epicatechin and epicatechin—(4—8)-epicatechin—(4—8, 2-O-7)-epicatechin. Peaks 3A and 3A' were likely these two trimers.

Two peaks of A-type tetramers 4A (28.7 min) and 4A' (31.6 min) were identified. Peak 4A gave rise to product ions m/z 863 and 575 due to QM cleavage of interflavan linkages, indicating a connection sequence of (epi)cat—(epi)cat—(epi)-cat—A—(epi)cat. This connection sequence was consistent with the absence of product ion m/z 573. Product ions m/z 861 and 573 derived from QM cleavage suggested that peak 4A' had a

connection sequence of (epi)cat—A—(epi)cat—(epi)cat—(epi)-cat. Peak 5A ($[M - H]^-$ m/z 1439.2) was identified as an A-type pentamer. Its product ions at m/z 575, 863, 1149, and 1151, due to QM cleavage of interflavan bonds, and m/z 861, from QM cleavage in A-type interflavan bond, indicated that this pentamer had a structure of (epi)cat—(epi)cat—(epi)cat—A—(epi)cat—(epi)cat. Peak 6A ($M - H]^-$ m/z 1725.4 was identified as a hexamer with two A-type interflavan linkages. We were not able to identify its connection sequence due to a large number of isomers and complexity of the mass spectra data. The content of procyanidin trimers, tetramers, pentamers, and hexamers in fraction II were 15.2%, 11.7%, 7.2%, and 5.3% (w/w), respectively.

Transport of Cranberry A-Type Procyanidins on Caco-2 Cells. A chromatogram of fraction I dissolved in HBSS is shown in Figure 2A. This solution was added into the apical side of
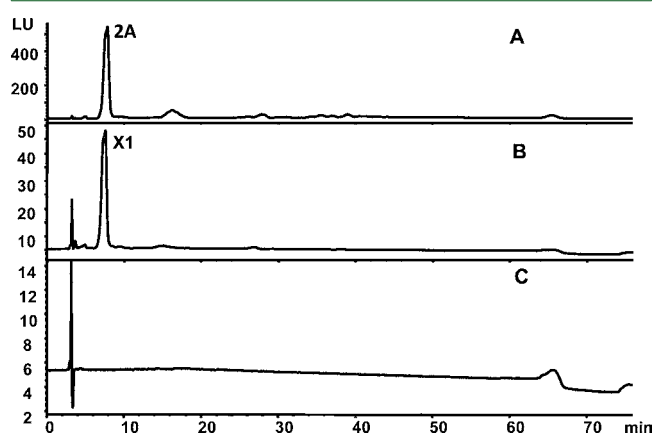

Figure 2. (A) HPLC chromatogram of fraction I in apical side at the beginning of transport experiment; peak 2A was A-type dimers. (B) HPLC chromatogram of HBSS from basolateral sides of Caco-2 monolayer after 2 h; peak X1 was detected in the HBSS solution from the basolateral side. (C) Chromatogram of control solution in the basolateral side.

Caco-2 cell membranes. After 2 h, the HBSS solution from the basolateral side of membranes was analyzed. Its chromatogram is depicted in Figure 2B. No peak was observed in the control sample where no procyanidins were added (Figure 2C). A peak X1 with retention time 7.5 min gave rise to m/z $[M - H]^-$ 575 with product ion at m/z 285 and 423 (Figure 3A).

Table 1. Identification of A-Type and B-Type Procyanidin Oligomers in Purified Procyanidin Fractions[a]

fraction	t_R (min)	peak label	connection sequence	$[M - H]^-$ m/z	product ions
I	8.2	2A	epicatechin-(2-O-7, 4—8)-epicatechin*	575	285(QM of A-type linkage), 423RDA,449HRF, 539(2 H₂O loss)
	17.4	3A	(epi)cat—(epi)cat—A—(epi)cat	863	423RDA, 449HRF, 575QM, 693(RDA, H₂O loss), 711RDA, 845(H₂O loss)
II	17.4	3A	(epi)cat—(epi)cat—A—(epi)cat	863	287QM, 575QM, 693(RDA, H₂O loss), 711RDA, 845(H₂O loss)
	21.6	3A'	(epi)cat—(epi)cat—A—(epi)cat	863	287QM, 575QM, 693(RDA, H₂O loss), 711RDA
	24.2	3B	(epi)cat—(epi)cat—(epi)cat	865	575QM, 577QM, 695(RDA, H₂O loss), 713RDA, 739HRF
	28.7	4A	(epi)cat—(epi)cat—(epi)cat—A—(epi)cat	1151	411HRF, 575QM, 863QM, 981(RDA, H₂O loss)
	31.6	4A'	(epi)cat—A—(epi)cat—(epi)cat—(epi)cat	1151	573QM, 691(RDA, H₂O loss), 861QM, 981(RDA, H₂O loss)
	36.0	5A	(epi)cat—(epi)cat—(epi)cat—A—(epi)cat—(epi)cat	1439	575QM, 693(RDA, H₂O loss), 861QM, 863QM, 1149QM, 1151QM
	39.8	6A	ND	1725	573QM, 691(RDA, H₂O loss), 711RDA, 861QM, 1151QM, 1435QM

[a]Symbol (epi)cat stands for catechin or epicatechin. RDA, retro-Diels—Alder cleavage; HRF, heterocyclic ring fission; QM, quinone methide cleavage. —A— denotes A-type interflavan linkage. *identified by comparing with standard. ND, unable to be determined.
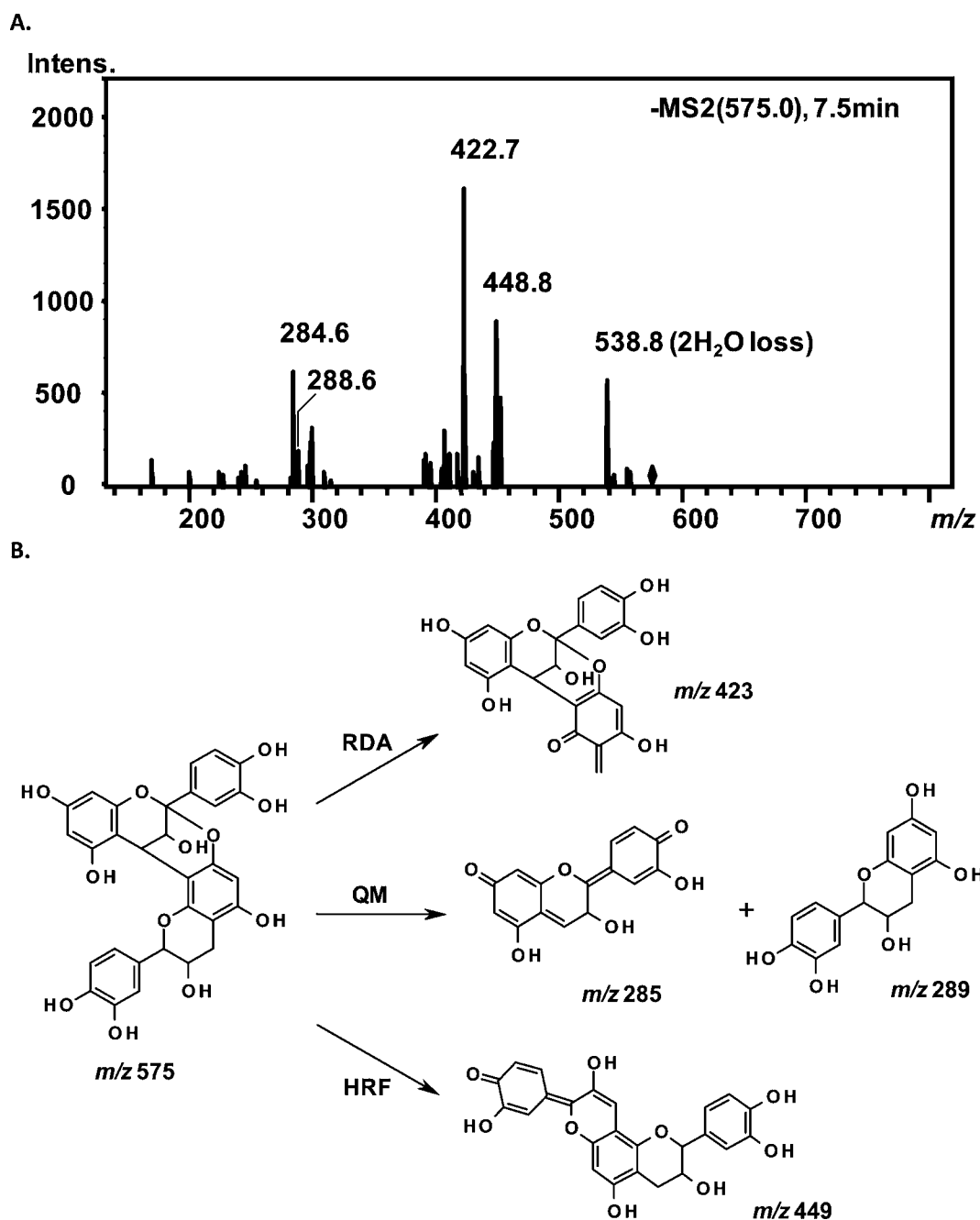
Figure 3. (A) Product ion spectrum of peak X1 and (B) postulated fragmentation pathway of procyanidin A2. RDA, retro-Diels−Alder cleavage; HRF, heterocyclic ring fission; QM, quinone methide cleavage.

This peak was identified as procyanidin dimer A2. The structure of A2 and its fragmentation pathway are depicted in Figure 3B.

The HBSS solution of fraction II, with the chromatogram shown Figure 4A, was applied on the apical side of differentiated Caco-2 cell monolayer. After 2 h, the HBSS solution from the basolateral side showed a chromatogram (Figure 4B) that was similar to that of Figure 4A. No peak of procyanidins existed in the control (Figure 4C). A peak (retention time 16.5 min, referred to as X2) showed m/z [M − H]$^-$ 863 with product ion m/z [M − H]$^-$ 575 and 693 (Figure 5A). This peak was the same as peak 3A in fraction II. It was an A-type trimer with a connection sequence of (epi)cat−(epi)cat−A−(epi)cat. Peak X3 (retention time 28.0 min) showed

m/z [M − H]$^-$ 1151 with product ion m/z [M − H]$^-$ 575 and 693 (Figure 5B). This peak was the same as peak 4A in fraction II. It was an A-type tetramer with a connection sequence of (epi)cat−(epi)cat−(epi)cat−A−(epi)cat. Identifications of other procyanidins in media of basolateral sides were inconclusive.

These results indicated that the A-type procyanidin dimers, trimers, and tetramers can traverse through the Caco-2 cell monolayer. These observations were consistent with the findings of Appeldoorn et al.,[14] who found that procyanidin dimers A1 and A2 were absorbable from the small intestine of rats during an in situ perfusion. However, they did not detect A-type trimers in rat blood, likely due to very low concentrations of A-type trimers in their testing samples.[14] Deprez et al.[12] also
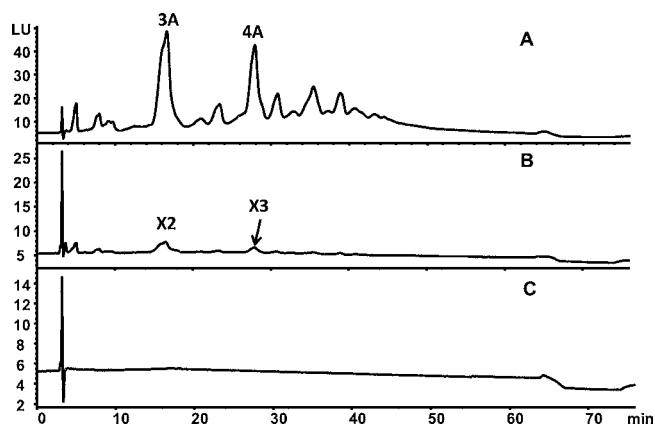
Figure 4. (A) HPLC chromatogram of fraction II in apical side at the beginning of transport experiment; peaks 3A and 4A were A-type trimers and tetramers. (B) HPLC chromatogram of HBSS from basolateral sides of Caco-2 monolayer after 2 h; peaks X2 and X3 were detected in the HBSS solution from the basolateral side. (C) Chromatogram of control solution in the basolateral side.
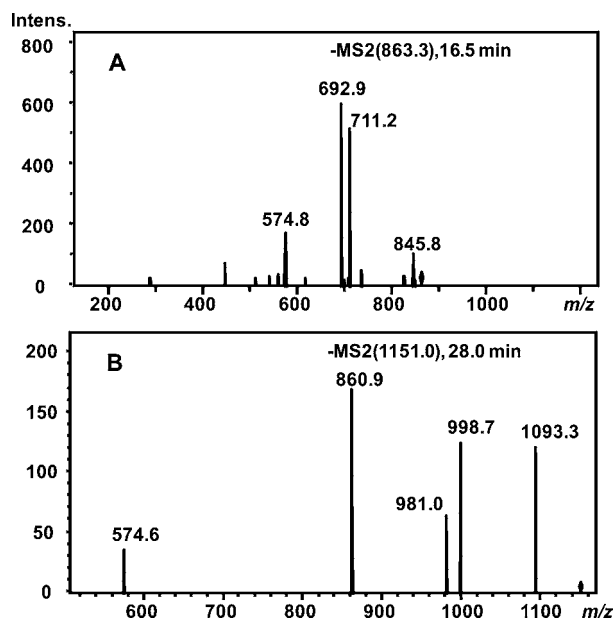

Figure 5. (A) Product ion spectrum of peak X2 and (B) product ion spectrum of peak X3.

showed that B-type procyanidin dimers, trimers, and tetramers were transported through Caco-2 membranes.

The P_{app} values, recovery rates, and transport ratios of A-type procyanidin dimers, trimers, and tetramers on this Caco-2 cell model are shown in Table 2. Recovery rates ranged from 93% for cranberry A-type tetramers to 104% for procyanidin A2 standard. Generally, the transport ratios were lower than 5%. This could be explained by Lipinski's "Rule of Five", which suggests that compounds with five or more hydrogen bond donors, or ten or more hydrogen bond acceptors, or molecular weight greater than 500 Da are usually poorly bioavailable due to their large apparent size.[22]

The transport ratio of A-type procyanidin dimer in fraction I was 0.6%, which was significantly lower than that of pure dimer standard A2, 4.8% ($p \leq 0.05$). Its permeability was ~8 times lower than that of the reported B3 trimer.[12] The HPLC chromatogram suggested the existence of other phytochemicals than procyanidin A-type dimer in this fraction. Other phytochemicals in fraction I could possibly interfere with transport of procyanidin dimer, thus decreasing the amount of dimer transported. Another possible reason for the discrepancy in transport ratio as well as P_{app} values is that procyanidins with high polymerization are astringent and may complex the membrane proteins and strengthen intercellular tight junctions.[12] This was confirmed by a lower transepithelial electrical resistance value of Caco-2 monolayers in procyanidin A2 standard transport experiments than those for fraction I (data not shown). A-type procyanidin dimer demonstrated a significantly higher transport ratio than B-type dimer (3.0%). This was in agreement with an early study which showed that A1 and A2 dimers were better absorbed than dimer B2 in the rat small intestine.[14] For the procyanidin A2 standard, its concentration in the basolateral side of Caco-2 cell monolayer after transport was about 14 μg/mL. This concentration is likely enough to exert antibacterial activity because an early previous study reported inhibition of *E. coli* adherence by cranberry procyanidins over a concentration range of 5 to 75 μg/mL.[23]

The permeability for A-type trimer was almost 10 times less than that of C2 trimer. And the A-type tetramer showed similar permeability with B-type polymer with a degree of polymerization of 6.[12] The transport ratios of trimer and tetramer were 0.4% and 0.2% respectively, which were significantly lower than that of dimers in fraction I ($p \leq 0.05$). The transport ratio decreased with an increase in molecular weight. Similar observations have been made by other researchers. For example, dimers were absorbed at a much lower efficacy than monomers.[14,24] Permeability of a procyanidin polymer with an average polymerization degree of 6 (molecular weight 1,740) on Caco-2 monolayers was approximately 10 times lower than (+)-catechin.[14] However, lower permeability of epicatechin than dimer and trimer standard

Table 2. Transport Ratio of A-Type Procyanidin Oligomers on Caco-2 Cells[a]

	sample added to apical side before transport (μg)	basolateral side after transport (μg)	apical side after transport (μg)	$P_{app} \times 10^{-6}$ (cm/s)	transport ratio (%)	recovery rate (%)
A-type dimer in fraction I	624.97 ± 12.75	3.66 ± 0.03	615.44 ± 16.21	0.26 ± 0.01 A	0.6 ± 0.0 A	99.1 ± 0.6
A-type trimer in fraction II	476.32 ± 20.17	1.75 ± 0.24	476.96 ± 1.31	0.16 ± 0.03 B	0.4 ± 0.1 B	100.5 ± 0.3
A-type tetramer in fraction II	529.96 ± 34.59	1.23 ± 0.039	493.31 ± 14.52	0.11 ± 0.01 C	0.2 ± 0.0 C	93.3 ± 2.7
(−)-epicatechin	383.80 ± 1.11	5.98 ± 0.06	375.33 ± 1.54	0.70 ± 0.01 c	1.6 ± 0.0 c	99.4 ± 0.1
procyanidin A2	744.06 ± 25.63	35.43 ± 0.22	736.06 ± 30.23	2.11 ± 0.05 a	4.8 ± 0.1 a	103.7 ± 7.6
procyanidin B2	946.29 ± 2.84	28.17 ± 0.94	905.82 ± 19.36	1.33 ± 0.04 b	3.0 ± 0.1 b	98.7 ± 1.9

[a]Data were expressed as the mean ± standard deviation ($n = 3$). Different letters in the same column indicate the significant differences in the mean at $p \leq 0.05$. Values with the same case were compared to each other.

has been observed in both a previous and current study.[14] This may due to an epicatechin specific efflux pump localized on apical membrane.[25]

In our study, a P_{app} value of 0.7×10^{-6} cm/s was observed for epicatechin. This value is similar to the 0.9×10^{-6} cm/s reported in a previous study.[12] The transport ratio of epicatechin (1.6%) was much lower than the values of 11.6% and 55.9% observed in jejunum and ileum using isolated rat small intestine perfusion model.[26] The different models employed may be the major reason for the distinct results. Our result on permeability of B-type dimer was consistent with a previous study on Caco-2 cells.[12]

This study showed that cranberry A-type procyanidin dimers, trimers, and tetramers traversed across Caco-2 cell monolayers with very low transport ratio. It suggested that these oligomers are absorbable in humans but their bioavailability is likely very low. This in part can explain the failure of detecting them in human blood. Appeldoorn et al.[14] reported that about 0.11% of A-type procyanidin dimers were absorbed after in situ intestinal perfusion in anesthetized rats for 30 min. The transport mechanism of procyanidin oligomers was likely permeation or diffusion, since no transporters have been identified that can transport epicatechin into intestinal epithelial cells. Absorbed epicatechin undergoes extensive phase II metabolism in the intestine, such as methylation, glucuronidation, and sulfation.[27] We did not detect any conjugates of A-type procyanidin dimers, trimers, or tetramers. This observation was consistent with that of Appeldoorn et al.,[14] who found that procyanidin dimers were not conjugated or methylated in rats. Another explanation could be that the levels of conjugates were below the detection limit of the HPLC−MS^n method.

In conclusion, our study found that A-type procyanidins dimers, trimers and tetramers from cranberry can be absorbed across Caco-2 cells although the transport ratios were strikingly low. It suggested that A-type procyanidin dimers, trimers, and tetramers are bioavailable in humans after cranberry consumption.

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Notes
The authors declare no competing financial interest.

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