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Effect of Lectins on the Transport of Food Factors in Caco-2 Cell Monolayers

YUMIKO OHNO, TAKAKO NAGANUMA, TOMOHISA OGAWA, AND KOJI MURAMOTO*

Department of Biomolecular Science, Graduate School of Life Sciences, Tohoku University, Sendai 981-8555, Japan

The effect of three plant lectins, soybean lectin (SBA), Japanese jack bean lectin (CGA), and wheat germ lectin (WGA), on the transport of various food factors, such as isoflavones, quercetin, dipeptides, and calcium ions, were investigated by use of an intestinal tract model, Caco-2 cell monolayers. The lectins increased the isoflavone transport but had no effect on aglycon transport. SBA increased the transport of quercetin glycosides, whereas CGA and WGA had no effect. The lectins increased the transport of calcium ions but showed no effect on the transport of dipeptides, carnosine, and anserine. Although SBA did not change the transepithelial electrical resistance (TER) value of the Caco-2 cell monolayers, CGA and WGA decreased the TER value. These results indicate that plant lectins affect the transport of food factors in different manners, presumably due to their specific sugar binding activity.

KEYWORDS: Caco-2; lectin; polyphenol; quercetin; isoflavone; carnosine; anserine; calcium; tight junction; intestinal transport

INTRODUCTION

Lectins, which are of nonimmune origin and are noncatalytic sugar-binding proteins, are widely distributed in the most common foods in varying amounts (*1*, *2*). Since many lectins, such as legume lectins, are relatively stable against heat denaturation and proteolytic digestion, the digestive tract is constantly exposed to biologically active lectins contained in fresh and processed foods. As a result, lectins interact with the epithelial surface of the digestive tract and cause physiological effects in human beings and animals, particularly when consumed in large quantities (*3*, *4*). In fact, it has been reported that people who consumed uncooked or partially cooked kidney beans suffered from food poisoning due to high doses of lectins (*5*). In experimental animals fed diets containing high levels of legume lectins, the evident symptoms are loss of appetite, decreased body weight, and eventually death (*6*). Consequently, lectins contained in foods are generally regarded as antinutritional factors together with enzyme inhibitors, though most of the adverse incidents were limited to legume lectins.

However, it is also true that low amounts of lectins may have some beneficial effects on biological systems (*7*). For example, some food lectins interact with particular bacteria to block them from attaching to the mucosa. Such lectins can be used to reduce the population of harmful commensal bacteria, prevent infection by pathogens, and promote the growth of beneficial microorganisms (*8*). Some lectins can influence the endocrine system with beneficial consequences for general metabolism by modulating the secretion of gut hormones (*9*).

Human adenocarcinoma cell lines, Caco-2 cells, derived from a colon carcinoma, have been used as in vitro models to estimate bioavailability of dietary components involving calcium ions (*10*−*13*), amino acids and peptides (*14*, *15*), and polyphenols (*16*−*20*). Caco-2 cells differentiate under standard culturing conditions to form confluent monolayers and acquire many features of absorptive intestinal cells during culture. Caco-2 cells spontaneously exhibit various enterocytic characteristics including the expression of brush border enzymes, nutrient transporters, and the formation of the intercellular tight junction (TJ) (*21*, *22*).

Dietary components have been found to affect the intestinal absorption of nutrients and other substances by modulating permeability (*23*−*27*). The modulating effects were further investigated in Caco-2 cell monolayers with the use of model compounds: lucifer yellow for the paracellular pathway, fluorescein for the monocarboxylic acid transporter-mediated pathway, and rhodamine 123 for the p-glycoprotein-mediated efflux pathway (*28*, *29*). Transepithelial electrical resistance (TER) is a highly sensitive parameter for membrane permeability. It is generally accepted that TER across Caco-2 cell monolayers is mainly determined by the ionic permeability of the intercellular junctions, which develop during postconfluent cell growth (*30*). Thus, the changing TER largely reflects an effect of TJ-mediated paracellular permeability, by which various hydrophilic compounds are transported.

* To whom correspondence should be addressed: tel/fax +81-22717-8807; e-mail muramoto@biochem.tohoku.ac.jp.

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In this study, the effect of several lectins, soybean lectin (SBA), Japanese jack bean lectin (CGA), and wheat germ lectin (WGA), on the transport of various food factors, such as isoflavones, quercetin, dipeptides, and calcium ions, were investigated by use of an intestinal tract model, Caco-2 cell monolayers. The effect of the lectins on the permeability of TJ was also examined by measuring TER value of the Caco-2 cell monolayers.

MATERIALS AND METHODS

Preparation of Food Factors. Soybean isoflavone concentrate was kindly supplied by Fuji Oil Co. (Osaka, Japan). The composition of the isoflavone was analyzed by reversed-phase high-performance liquid chromatography (HPLC) (*31*, *32*) with authentic isoflavones (Fujicco, Kobe, Japan). The isoflavone was dissolved in 70% aqueous ethanol (0.1 mg/mL) and subjected to HPLC analysis on a Capcelpak ODS AG120 (4.6 × 250 mm) (Shiseido, Tokyo, Japan) at a flow rate of 1.0 mL/min at 40 °C. Solvent A was 0.1% acetic acid in water, and solvent B was 0.1% acetic acid in acetonitrile. A linear gradient elution was carried out from 10% to 20% solvent B for 20 min and then from 20% to 30% solvent B for another 40 min. Detection was made by UV absorbance at 254 nm. The isoflavone contained 28.0% daidzin, 34.3% glycitin, 14.2% genistein, 4.8% acetyl daidzin, and 2.4% acetyl genistein. An isoflavone solution (80 mM in dimethyl sulfoxide) was diluted with Hanks' balanced salt solution (HBSS) (pH 7.3) (Sigma Japan, Tokyo, Japan) to give 80 μM (final concentration of dimethyl sulfoxide 0.1%) and used for transport experiments.

Isoflavone aglycons were prepared by β-glucosidase treatment of isoflavones (*33*). An isoflavone solution (0.2 mg/0.1 mL in 70% ethanol) was mixed with 0.77 mL of 5 mM sodium acetate buffer (pH 5.0) and reacted with 0.13 mL of β-glucosidase (from almond, 32 units/mL) (Wako Chemical, Osaka, Japan) for 5 h at 50 °C. HPLC analysis showed that the reaction mixture contained 1.9% daidzin, 2.9% glycitin, 10.7% genistein, 6.0% acetyl daidzin, 4.0% acetyl genistein, 28.7% daidzein, 22.4% glycitein, and 3.5% genistein. The mixture was then diluted with HBSS (pH 7.3) and used for transport experiments.

Quercetin glycosides were prepared from fresh onion. About 80 g of the edible portion was homogenized in methanol (180 mL) and centrifuged at 4000*g* for 20 min at 4 °C. The extract was concentrated by a rotary evaporator and dried in vacuo to give about 7 g of crude quercetin glycosides. This extract was then analyzed by reversed-phase HPLC (*34*) on a Capcelpak ODS AG120 column (4.6 × 250 mm) with authentic quercetin (Wako Chemical). Solvent A was 0.1% trifluoroacetic acid (TFA) in water, and solvent B was 0.1% TFA in acetonitrile. A linear gradient elution from 15% to 40% solvent B was carried out for 20 min at 1.0 mL/min at 40 °C. The crude quercetin glycosides containing quercetin 3,4′-diglucosides (Q3,4′G) (3.6 nmol/mg) and quercetin 4′-monoglucoside (Q4′G) (4.0 nmol/mg) were dissolved in HBSS (10 mg/mL) to give 36.0 μM Q3,4′G and 40.6 μM Q4′G for transport experiments.

Dipeptides, carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-1-methyl-L-histidine), were gifts from Yonezawa Hamari Chemicals (Osaka, Japan) and Yaizu Suisan Kagaku Industry (Shizuoka, Japan), respectively. The mixture containing 1 mM carnosine and anserine was used for transport experiments. The dipeptides were analyzed by an amino acid analyzer (Shimadzu, Kyoto, Japan) on a Shim-Pack Amino-Li column (Li type, 6.0 × 100 mm) using the postcolumn labeling method with o-phthalaldehyde.

Preparation of Lectins. Soybean (*Glycine max*, Miyagishirome) was obtained from a local farmers' cooperative. Japanese jack bean (*Canavalia gladiata*) and wheat germ were kindly supplied by Yoshitome Sangyo (Tokyo, Japan) and Nisshin Seifun (Tokyo, Japan), respectively. Soybean lectin (SBA) (*35*), Japanese jack bean lectin (CGA) (*36*, *37*), and wheat germ lectin (WGA) (*38*) were isolated by ammonium sulfate precipitation and affinity chromatography on cross-linked guar gum gel, Sephadex G-100, and chitin beads, respectively. Each lectin fraction was dialyzed against distilled water and lyophilized and was kept at −20 °C until use. Purity of the lectins was examined by SDS−polyacrylamide gel electrophoresis.

Transport Experiments. A Caco-2 cell line was obtained from Dainippon Pharmaceutical (Osaka, Japan). Minimum essential medium Eagle (MEM) and fetal bovine serum were purchased from Sigma Japan. The cells were cultured in MEM supplemented with 10% fetal bovine serum in the presence of 100 units/mL penicillin, 0.1 μg/mL streptomycin, and 0.25 μg/mL amphotericin β. The monolayer cultures were maintained at 37 °C grown in 5% CO_2. The cells were subcultured at 70−80% confluency. Caco-2 cells were seeded on Transwell inserts (polycarbonate membrane, 0.4 μm pore size, Corning Costar Japan) in 24-well plates at a density of 1.5×10^5 cells/insert (0.33 cm^2). The basolateral and apical compartments contained 0.1 and 0.6 mL of culture medium, respectively. Culture medium was replaced twice a week. Cell monolayers cultured for 13−17 days were used for transport experiments. All experiments were performed at cell passages 20−40.

The cell monolayers on Transwell inserts were rinsed twice with transport buffer (HBSS) (pH 7.3) and incubated in the buffer for 60 min at 37 °C. Food factors and lectins (0−180 μg/mL) were dissolved in HBSS and sterilized by filtration. The test solutions (0.2 mL) containing food factors and lectins were added into the apical side of inserts. After incubation for 2−4 h at 37 °C, the apical and basolateral solutions were further analyzed. Isoflavones and quercetin glycosides were determined by reversed-phase HPLC as described above. Carnosine and anserine were analyzed by an amino acid analyzer.

For calcium transport experiments, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid- (HEPES-) buffered saline solution (pH 7.4) (HSS) was used with (+) or without (−) 5 mM $CaCl_2$ instead of HBSS. HSS was composed of 20 mM HEPES, 120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, and 10 mM D-glucose. HSS (+) and HSS (−) were added to the apical and basolateral compartments, respectively. The calcium concentration was determined on an atomic absorption spectrophotometer (AA-6800, Shimadzu).

Transepithelial Electrical Resistance Measurement. The cells were seeded on Transwell inserts (Corning Costar) in 12-well plates at a density of 5.0×10^5 cells/insert (1.0 cm^2). The medium was changed twice a week. The integrity of the monolayer was accomplished after 13−17 days. The integrity of the cell monolayer was evaluated by measuring TER with Millicell-ERS equipment (Millipore, MA). The cell monolayers showing TER values of more than 350 $\Omega \cdot cm^2$ were used for the experiments. The monolayers were rinsed gently three times with HBSS and equilibrated with the same buffer for 30 min at 37 °C. The TER of the monolayer was measured before and after the assay sample was added to the insert.

Cytotoxicity of Lectins. Caco-2 cells were seeded in a 96-well microtiter plate at a density of 1.0×10^4 cells/well. These cells were cultured for 13−17 days before experiments. Lectins were dissolved in MEM and added to the cells at a final concentration of 12.5−200 μg/mL. After removal the culture medium by aspiration, 100 μL of lectins dissolved in MEM was added to the cells and incubated for 4 h. Cytotoxicity was measured by WST-1 assay kit (Dojindo, Kumamoto, Japan). The WST-1 reagent was incubated with the cells for another 3 h, and then the absorbance at 450 nm was measured by a titer plate reader (Sanko, Tokyo, Japan).

RESULTS

Effect of Lectins on Isoflavone Transport. Isoflavone glycosides (28.2 μmol/L daidzin, 34.6 μmol/L glycitin) were added to the apical side of Caco-2 cell monolayers in the presence or absence of the lectins SBA, CGA, and WGA. Isoflavone glycosides could not be detected in the basolateral side in the absence of lectin after 4-h incubation. By the addition of the lectins, the isoflavones began to be transported to the basolateral side in a dose-dependent manner (**Figure 1**). At 18 μg/mL, the transported isoflavones increased in the following order: SBA < WGA < CGA. The lectins showed no apparent cytotoxicity against Caco-2 cells at least up to 200 μg/mL under the conditions tested in this study. SBA showed a maximal transport of the isoflavones at 90 μg/mL and the least transport at 180 μg/ mL. The transport increased with time (**Figure 2**),

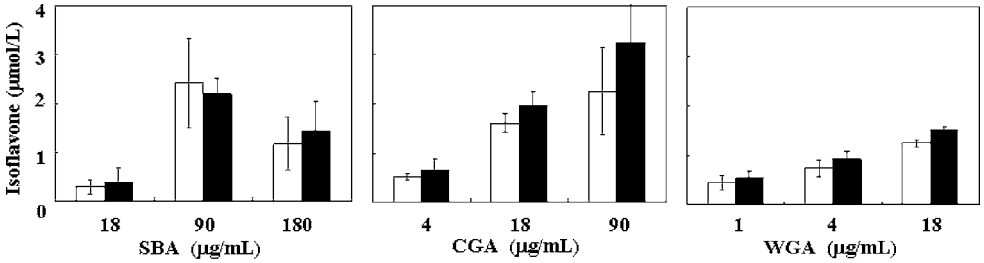

Figure 1. Effect of lectins on isoflavone transport across Caco-2 cell monolayers. Isoflavone concentrations in the basolateral side were measured after 4-h incubation of the monolayers with isoflavones (80 μM) in the presence of lectins. Open bars and solid bars indicate the values of daidzin and glycitin, respectively, as the mean ± SE ($n = 6$). In the absence of lectin, isoflavones could not be detected on the basolateral side.

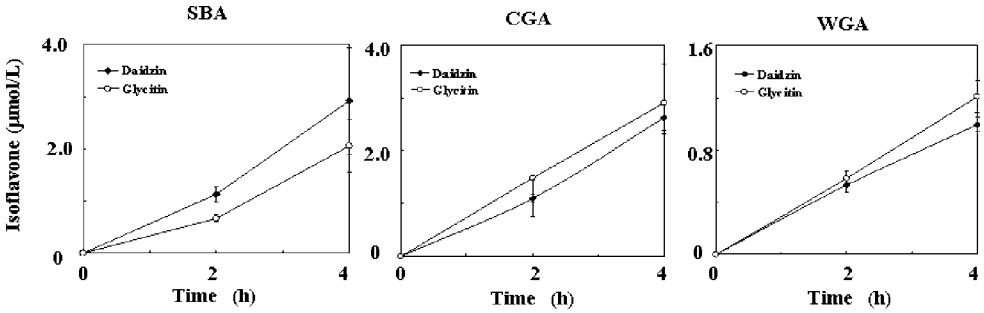

Figure 2. Time-course plots of isoflavone transport across Caco-2 cell monolayers in the presence of lectins. The monolayers were incubated with isoflavones (80 μM) in the presence of SBS (90 μg/mL), CGA (90 μg/mL), and WGA (18 μg/mL) for the indicated periods, and the isoflavone concentrations on the basolateral side were measured. Values are expressed as the mean ± SE ($n = 6$).

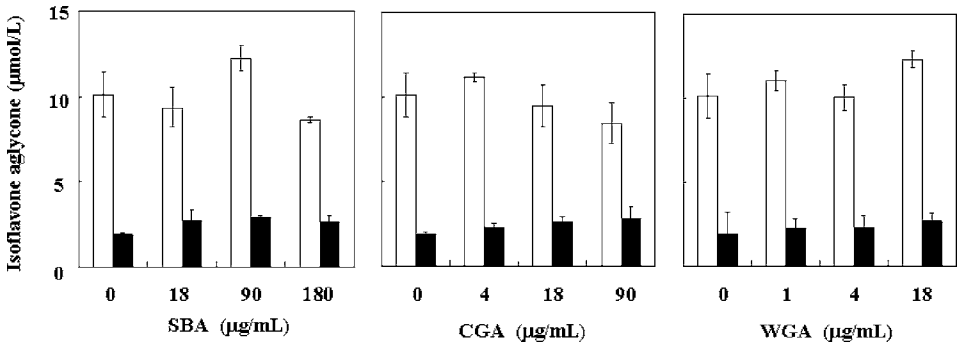

Figure 3. Effect of lectins on isoflavone aglycon transport across Caco-2 cell monolayers. Isoflavone aglycon concentrations on the basolateral side were measured after 4-h incubation of the monolayers with isoflavone aglycons (80 μM) in the presence of lectins. Open bars and solid bars indicate the values of daidzein and glycitein, respectively, as the means ±SE ($n = 6$).

and about 4−8% of the initial amount of isoflavone glycosides was transported to the basolateral side after 4-h incubation.

In contrast to isoflavone glycosides, isoflavone aglycons were transported through the monolayers in the absence of lectins (**Figure 3**). The aglycon transport did not increase in the presence of lectins, and the daidzein transport even slightly decreased in the presence of 18−90 μg/mL CGA.

Effect of Lectins on the Transport of Other Food Factors. Quercetin glycosides, Q4′G and Q3,4′G, were minimally transported across Caco-2 cell monolayers (**Figure 4A**). In the presence of 90 μg/mL SBA and CGA, Q4′G but not Q3,4′G was transported at a level 3 times greater than that observed without lectins. However, WGA did not show such an effect. Lutin, a diglycoside, was not transported in the presence of lectins (data not shown).

The amounts of carnosine and anserine transported to the basolateral sides were assessed in the presence and absence of lectins as shown in **Figure 4B**. A significant amount (34.8 ± 9.3 μmol/L) of the dipeptides was transported after 2-h incubation without lectins, and the amount did not increase with lectins.

To investigate calcium ion transport, HSS (+) and HSS (−) were added to the apical and basolateral compartments, respectively, and the calcium ions in the basolateral sides were quantitatively analyzed after 4-h incubation. As shown in **Figure 4C**, the lectins increased the transport of calcium ions.

Effect of Lectins on TER Values. TER measurement can be used as an indicator of the tightness of intercellular junctions: a decrease in TER indicates an increase of the paracellular permeability, or vice versa. The effect of lectins on TER values of the cell monolayer is shown in **Figure 5**. Incubation with CGA (90 μg/mL) or WGA (90 μg/mL) for 4 h significantly decreased the TER values, whereas no significant difference in the TER value was observed with SBA (90 μg/mL).

The effects of lectins on TER values were examined in the presence of various food factors (**Figure 6**). CGA and WGA decreased the values in the presence of isoflavones and quercetin glycosides compared to the values in their absence. These lectins did not affect the values greatly in the presence of dipeptides or calcium ions. The addition of quercetin glycosides increased TER values. Although a slight decrease of TER values was detected in the presence of SBA, the lectin did not decrease the

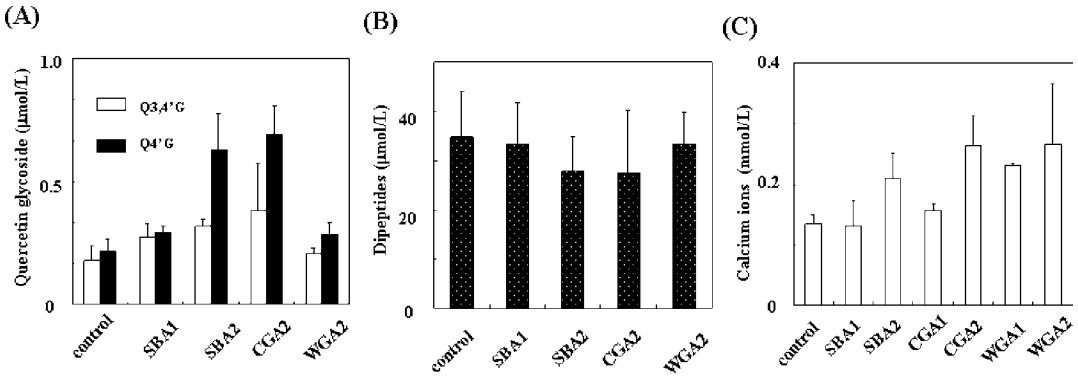

Figure 4. Effect of lectins on the transport of quercetin glycosides, dipeptides, and calcium ions across Caco-2 cell monolayers. Quercetin glycoside (**A**) and dipeptide (**B**) concentrations in the basolateral side were measured after 2-h incubation of the monolayers with quercetin glycosides (36.0 μM Q3,4′G and 40.6 μM Q4′G) and dipeptides (1 mM), respectively, in the presence of lectins. For calcium transport, the monolayers were incubated with 5 mM calcium ions (**C**) for 4 h. SBA1, 18 μg/mL; SBA2, 90 μg/mL; CGA1, 18 μg/mL; CGA2, 90 μg/mL; WGA, 18 μg/mL; WGA2, 90 μg/mL. Values are expressed as the means ± SE ($n = 6$).

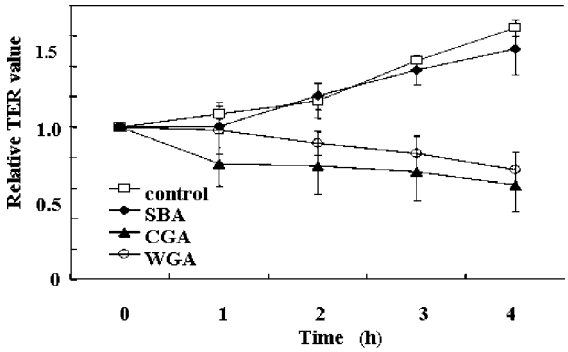

Figure 5. Effect of lectins on TER of Caco-2 cell monolayers. TER of the monolayers was measured after incubation with lectins (90 μg/mL) for the indicated periods. Values are expressed as the means ± SE ($n = 6$).

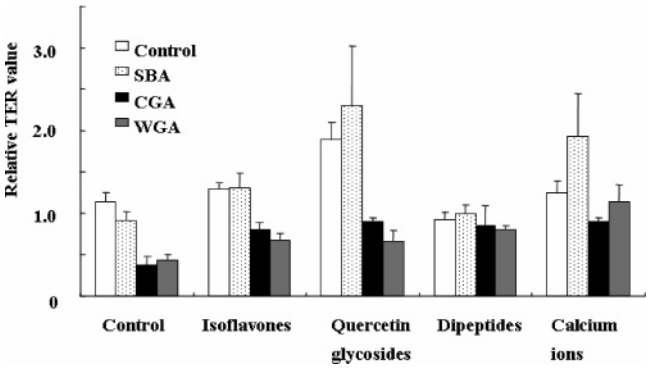

Figure 6. Effect of lectins on TER of Caco-2 cell monolayers in the presence of food factors. TER of the monolayers was measured after 4-h incubation with lectins (90 μg/mL) in the presence of 80 μM isoflavones, 80 μM quercetin glycosides, 1 mM dipeptides, and 5 mM calcium ions, respectively. Values are expressed as the means ± SE ($n = 6$).

values in the presence of various food factors but slightly increased the values with quercetin glycosides or calcium ions.

Inactivated lectins, which had been treated by heating at 100 °C for 30 min, did not affect either the transport of food factors or TER values of Caco-2 cell monolayers.

DISCUSSION

Absorption of nutrients and food factors across the intestinal epithelium may occur by one or more different transport pathways, such as passive paracellular transport, passive transcellular transport, and carrier-mediated transport (*39*). Lipophilic compounds are rapidly transported by passive transcellular pathways. A number of carriers are expressed in the small intestinal mucosa, which is responsible for the absorption of substrates and their analogues. It is generally assumed that hydrophilic compounds are transported across the intestinal epithelium through the paracellular pathway. The apical and basolateral sides are separated by tight junctions, providing a seal between adjacent epithelial cells that restricts transport by this pathway. Tight junctions are modulated in permeability by various factors, resulting in a highly dynamic transport pathway (*40*).

Caco-2 cell lines are routinely cultivated as monolayers on permeable filters for studies of the transepithelial transport of food constituents and drugs (*41*). The cells exhibit morphological as well as functional similarities to intestinal enterocytes such as microvillus structure, tight junctions, and brush border enzymes. Many active transport systems for nutrients and food factors found in small intestinal enterocytes are also expressed in Caco-2 cells (*21*, *22*). Hence, Caco-2 cell monolayers have been preferably employed as the transepithelial transport model systems to explore modulating factors. It has been found that capsianoside (*30*) and vitamin D (*42*) reduce the TER and increase the permeability of the Caco-2 cell monolayer. Tea polyphenols inhibit the transport of dietary phenolic acids mediated by the monocarboxylic acid transporter (*28*, *29*).

The present study showed that SBA, WGA, and CGA had a modulating effect on the transport of various food factors. WGA and CGA caused a decrease of TER values, indicating an increase of the paracellular transport. The three lectins increased the transport of isoflavones and quercetin glycosides but not of aglycons. The possible involvement of the sodium-dependent glucose transporter (SGLT-1) in the absorption of polyphenolic compounds has been reported (*43*). The transport of dipeptides, which might be conducted by the peptide transporters 1 (PepT1), was unaffected by the lectins (*44*). The present study suggests that the lectins tested modulate SGLT-1. The major route of calcium transport across Caco-2 cell monolayers has been shown to be the paracellular pathway (*45*). This is in agreement with the present result, that WGA and CGA increased the calcium transport with a decrease of the TER values. SBA showed only a small increase in calcium transport without TER change, suggesting that lectin had a weak effect on tight junctions (**Figure 5**).

Lectins are found in every organism, many of which are used as foods. Each lectin has characteristic properties, such as sugar

specificity, stability against heating or protease digestion, etc. SBA, CGA, and WGA have sugar-binding specificities for *N*-acetyl-D-galactosamine/D-galactose, D-mannose, and *N*-acetyl-D-glucosamine, respectively; therefore, each lectin interacts with its specific binding sites on the surface epithelium of the digestive tract. The lectins showed no binding affinity toward either isoflavone or quercetin glycosides as was predicted by their sugar specificity. CGA has properties similar to those of concanavalin A (ConA) from jack beans. The precise mechanism of the lectins to modulate the permeability of Caco-2 cell monolayers still remains to be solved. It is known that some hormones regulate the TER of cell monolayers (*46*). If it is taken into account that WGA and ConA can interact with the insulin and epidermal growth factor receptor (*47*, *48*), it may be possible that the lectins modulate tight junctions by interacting with hormone receptors. ConA has been also shown to interact with sealing proteins of the tight junctions (*49*).

Although animal experiments have shown that lectins damage the gut and lead to various nutritional disorders, this generally only occurs when large amounts of some lectin are eaten. The present study demonstrated that several lectins could increase the permeability of some food factors without damaging Caco-2 cell monolayers. Recently, we found that major proteins of yam tubers *Dioscorea batatas* were composed of mannose-binding and maltose-binding lectins (*50*). The yam tubers are generally consumed without cooking in Japan and are used as herbal medicines in East Asia. The lectins contained in the yam affected the permeability of Caco-2 cell monolayers without changing TER values (unpublished data). Since lectins are contained in most foods, it would be possible to regulate the transport of nutrients and food factors with appropriate choice of foods containing lectins.

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