Binding and Transport of Some Bioadhesive Plant Lectins Across Caco-2 Cell Monolayers

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INTRODUCTION

The interaction between plant lectins and intestinal epithelial cells is of interest for various reasons. First, a variety of plant lectins is part of the regular diet of humans and farm animals (1). Some of these lectins, such as phytohemagglutinin from *Phaseolus vulgaris*, may cause an acute intestinal syndrome with nausea, vomiting, and diarrhea, if kidney beans are consumed raw or poorly cooked (2,3). Other lectins, such as tomato lectin from *Lycopersicum esculentum*, are apparently nontoxic. This lectin is ingested with the consumption of raw tomatoes in relatively large amounts (100–200 mg a year, (4)) and has been found to bind to the intestinal villi without deleterious effects (5). Second, there is interest in lectins in the context of drug delivery with the aim of improving the oral bioavailability of poorly absorbed drugs, such as peptides and proteins, through a prolonged and/or intensified contact to the intestinal mucosa. Nontoxic lectins can possibly be used to achieve specific binding of such systems to the epithelial cell surface (6). In addition, tomato lectin has also been reported to cross the tissue of rat everted intestinal sacs *in vitro* by a mechanism involving adsorptive endocytosis (7). After feeding of radiolabeled phytohemagglutinins to mice, a relatively high fraction (5–10%) of the dose appeared in the blood, and it has been suggested that lectins may be used to enhance the absorption of macromolecular drugs or drug carriers from the digestive tract (8). In this study, we compared bioadhesion of the aforementioned lectins and bovine serum albumin (BSA) with their transport across filter-grown monolayers of the human colon carcinoma cell line Caco-2 (9). These monolayers have tight junctions and resemble structurally and functionally the intestinal epithelium, making them a suitable model for studying drug transport phenomena under controlled conditions *in vitro* (10,11). In this study, we did not observe significantly enhanced transport of bioadhesive lectins due to adsorptive endo-/transcytosis in comparison with the transport of a nonadhesive protein (albumin) by fluid-phase transcytosis.

MATERIALS AND METHODS

BSA (fraction V powder; 70 kDA) was purchased from Sigma (St. Louis, MO), tomato lectin (TL; 71 kDa) from Vector (Burlingham, CA), and phytohemagglutinin L_4 (PL; 117 kDa) and phytohemagglutinin E_4 (PE; 117 kDa) from EY-Labs (San Mateo, CA). The (glyco)proteins were labeled with ^{125}I as described previously (6). Free label was removed by gel chromatography (Econo-Pac 10 DG desalting columns, Bio-Rad), followed by repeated ultrafiltration [Ultrafree-MC, low-binding PLGC (10,000 NMWL) membrane, Millipore] immediately prior to the experiments and until the radioactivity in the filtrate was less than 2% of the total radioactivity. ^{3}H-Mannitol (ICN, Irvine, CA) was evaporated to dryness under a nitrogen stream to remove volatile tritium immediately prior to each experiment. HRP (type VIa) was purchased from Sigma.

Caco-2 cells (passages 25–46) were a gift from Dr. W. C. Shen (University of Southern California, Los Angeles) and kept in culture as previously described (6). Cells were seeded (60,000 cells/cm^2) on collagen-coated Transwell-COL, 0.4-μm-pore size tissue culture inserts (Costar, Cambridge, MA). Confluence was verified by phase-contrast microscopy and by measuring the transepithelial electrical resistance (TEER) using an electric volt/ohm meter (EVOM, World Precision Instruments, Sarasota, FL) with a pair of chopstick electrodes. Monolayers were used for experiments 21 ± 2 days after seeding, the TEER being in a range between 150 and 200 Ω · cm^2 after subtracting the resistance of blank filters.

For transport and binding studies, Caco-2 cell monolayers were grown on 24.5-mm transwells in six-well plates (surface, 4.71 cm^2; apical volume, 1.5 mL; basolateral volume, 2.5 mL). Monolayers were first equilibrated with glutathione–bicarbonate Ringer's solution [GBR (12)] containing 0.5% BSA for 30–60 min at the temperature of the experiment. To study the transport of iodinated BSA, BSA-free GBR was used. Either 150 μL (apical) or 250 μL (basolateral) buffer, respectively, was removed and replaced by the same volume of a donor stock solution, containing radiolabeled (glyco)protein in GBR. Concentrations were chosen to yield a final concentration in the donor compartments of 43 n*M* for all (glyco)proteins (i.e., 3 μg/mL for TL or BSA and 5 μg/mL for PL and PE), corresponding to 10^6 cpm/mL radioactive tracer. To study concentration dependence of binding and transport, the three lectins were also used at a 430 n*M* concentration, and BSA in GBR containing 0.5% BSA. Every hour, TEER was measured, and a sample (1.0 mL) taken from the receptor compartments and replaced by fresh GBR (0.5% BSA). At the end of the experiment, the monolayers were washed quickly with ice-cold saline and lysed in 0.3 *N* NaOH/1% Triton X-100 for 15 min at 37°C to count cell-associated radioactivity.

Lectin effects on the barrier properties of Caco-2 cell monolayers were studied in 12-mm Transwells in six-well plates (surface, 1.13 cm^2; apical volume, 0.6 mL; basolateral volume, 1.0 mL), all from the same passage. After 1 hr of

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incubation at 37°C with GBR, containing 0.5% BSA and 1 m*M* mannitol, TEER was measured and an appropriate volume in either compartment was replaced by a lectin stock solution. For the control experiments, this step was omitted. Subsequently, 60 or 100 μL, respectively, of the lectin-containing compartment was replaced by the same volume of a stock solution of the marker molecules, yielding a final donor concentration of 1 mg/mL HRP and 10^6 cpm/mL ^{3}H-mannitol. Incubation was continued at 37°C. ^{3}H-Mannitol was measured in hourly samples (50 μL) by scintillation counting. Another 50 μL was assayed for HRP activity by the *o*-dianisidine colorimetric method (13). At the end of the experiment, TEER was measured again, and the monolayers were washed and lysed in 1.0 mL 0.1% Triton X-100 in water (14) to determine the amount of cell associated radioactivity and HRP activity.

RESULTS AND DISCUSSION

The amount of BSA or lectins which was bound to or internalized by Caco-2 cells after 5 hr under various conditions is shown in Fig. 1. In contrast to the nonadhesive BSA, the three lectins showed remarkable bioadhesion to the apical and to the basolateral side of Caco-2 cell monolayers. The affinity was ranked PE > PL ~ TL, and the affinity to the apical side was about 1.5–2 times stronger than to the basolateral side. In the presence of a 10-fold excess of unlabeled lectin, the amount of cell-associated radioactivity was reduced by approximately 50%, indicative of specific, saturable lectin–cell interaction. Lowering the temperature to 5°C at 43 n*M* significantly reduced bioadhesion of PE and TL, but not of PL, indicating that cell interaction of the first two lectins involves at least partly a temperature-dependent process such as endocytosis.

Transport of BSA and lectins across Caco-2 cell monolayers in the same experiments in terms of total radioactivity (i.e., intact plus degraded proteins) was very low (Fig. 2). Unlike binding/uptake, transport of the four macromolecules was very similar and symmetric, with some exception for PE. While the transport of all other components was linear, with an apparent permeability of the order of 10^{-8} to 10^{-7} cm/sec, PE showed exponentially increasing transport with time. Furthermore, transport of this lectin was asymmetric, the area under the transport–time curve (AUC) being about two times larger in the basolateral-to-apical (B → A) than in the apical-to-basolateral (A → B) direction, and showed a slightly significant ($P < 0.1$) inhibition of about 50% in both directions by a 10-fold excess of unlabeled lectin.

The three- to fivefold reduction of transport at 5°C (Fig. 2) and the almost-complete degradation of the radioactive tracer in the receiver samples (trichloroacetic acid precipitation; data not shown) are indicative of a transcytotic process. Surprisingly, lectin transport was far less dependent on binding to the cell surface than expected and must have occurred predominantly by fluid-phase rather than adsorptive transcytosis. A certain adsorptive component was observed only for PE transport, but the total amount of transcytosed PE (AUC) was not much higher than that of the other three macromolecules (Fig. 2).

Such apparent uncoupling between surface binding and transcytosis has been reported by Ryser *et al.* (15) in the interaction of heparin with cultured cells. Apparently, extracellular heparin binds to a network of surface macromolecules (fibronectin) which do not participate in membrane vesiculation, thereby preventing rather than enhancing endocytosis of extracellular ligands. By analogy, lectins may be recognized by specific oligosaccharide sequences in the glycocalyx of epithelial cells (16) and are prevented from being internalized. Nevertheless, the temperature-sensitive cell association of TL and PE, but not of PL (Fig. 1), indicates that some of the lectins did undergo adsorptive endocytosis. The ligands might remain bound to the membrane and are not released or they may be recycled back to the cell surface rather than being transcytosed.

The lectin effects on the barrier properties of Caco-2 monolayers are shown in Table I. Transport of mannitol, which was symmetrical, with an apparent permeability coefficient of about 10^6 cm/sec, did not show any significant deviations from the control treatment under the influence of any lectin at any concentration or site of application. Cell-associated radioactivity, indicating binding or uptake by the cells, was negligible in all cases (not shown). In contrast, transport of biologically active HRP was barely detectable in the A → B direction, but 10–100 times more and exponen-

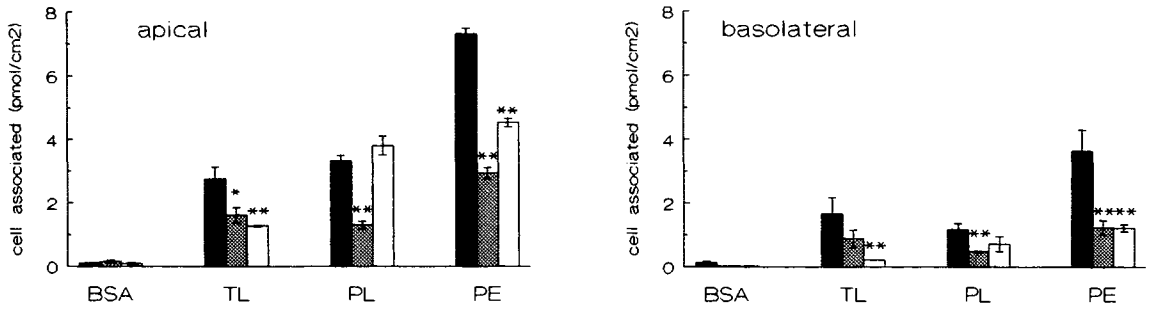

Fig. 1. Binding/uptake of radiolabeled (glyco)proteins by Caco-2 cell monolayers. Bars represent mean ± SD (n = 6–9). Black bars—reference (43 n*M*, 37°C); hatched bars—10-fold excess of unlabeled lectin, 37°C; open bars—43 n*M*, 5°C. Each macromolecule was studied in separate experiments, all treatments in triplicate, using up to 18 monolayers (three plates) of the same passage at a time. Left: Incubation of the apical side. Right: Incubation of the basolateral side. * and ** indicate significance at $P < 0.1$ or $P < 0.05$, respectively (nonparametric U test). For basolateral TL, inhibition by a 10-fold excess of cold lectin failed to reach statistical significance due to relatively large passage-to-passage variations, but significant inhibition was always observed within the same experiment.

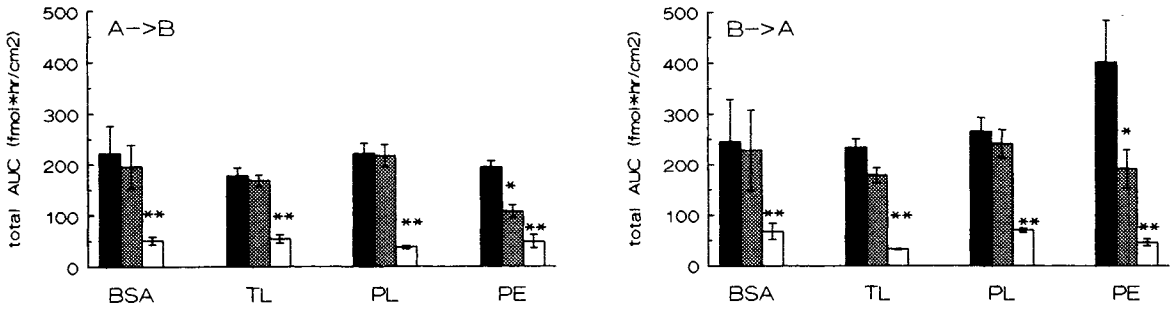

Fig. 2. Transport of radiolabeled glycoproteins across Caco-2 cell monolayers. Left: Apical-to-basolateral (A → B) direction. Right: Basolateral-to-apical (B → A) direction. For details see the legend to Fig. 1.

tially increasing with time in the B → A direction. This finding is consistent with that of Heyman *et al.* (17). At the end of the 5-hr experiment, much more HRP activity was found associated with the cells after exposure to the basolateral side than to the apical side. In the presence of 50 μg/mL (430 n*M*) PE in the basolateral donor compartment, transport of HRP in the B → A direction started to increase dramatically after about 2 hr of incubation (not shown). Simultaneously, a significant drop in TEER was noticed at the end of the experiment, indicating lowering of the barrier function of the monolayer. The same concentration of PE which induced these effects (Table I) competitively inhibited the transport of radioactive PE tracer (Fig. 2). A correlation between lectin binding and extent of lectin-induced changes in the cellular morphology and metabolism of Caco-2 cells has been reported earlier (18,19), including effects on the actin cytoskeleton which lead to a shortening of microvilli (20). Because the cytoskeleton is also intimately associated with the tight intercellular junctions (21), it may be speculated that such lectins increase the paracellular permeability of the intestinal epithelium. Depending on the dose and length of exposure, they may eventually promote their own absorption along such a route, with only a minor amount undergoing transcytosis.

In summary, the three lectins investigated showed only a marginally, if any, enhanced transcytosis across Caco-2 cell monolayers, in spite of specific binding and partial internalization by these cells. As far as this model is representative of the native intestinal mucosa, the reported relatively high oral bioavailability of some toxic lectins is probably due

Table I. Effect of Lectins on Barrier Properties of Caco-2 Cell Monolayers[a]

	Apical to basolateral				Basolateral to apical			
		HRP		TEER (% of		HRP		TEER (% of
	Mannitol, P_{app} (cm/sec) * 10^7	Bound (ng/cm^2)	AUC (ng/cm^2 * hr)	initial value)	Mannitol, P_{app} (cm/sec) * 10^7	Bound (ng/cm^2)	AUC (ng/cm^2 * hr)	initial value)
Control	11.5	262	12.4	102.5	7.9	1540	52.0	82.2
	(0.7)	(73)	(8.0)	(4.0)	(0.1)	(180)	(5.3)	(4.0)
TL								
43 n*M*	12.0	90	2.4	109.6	11.6	1489	74.5	113.4
	(0.1)	(15)	(1.7)	(4.1)	(0.3)	(1064)	(7.6)	(2.7)
430 n*M*	10.4	128	4.5	117.3	11.4	908	68.6	105.0
	(0.3)	(53)	(1.4)	(2.7)	(0.2)	(300)	(2.9)	(9.6)
PL								
43 n*M*	13.5	198	18.3	88.5	8.5	1097	76.5	81.7
	(0.6)	(47)	(2.2)	(0.4)	(0.04)	(165)	(16.8)	(2.3)
430 n*M*	11.6	214	18.0	85.6	8.9	1020	48.4	78.0
	(0.4)	(43)	(1.7)	(6.2)	(0.03)	(342)	(6.2)	(4.1)
PE								
43 n*M*	11.8	274	13.5	87.0	8.7	1405	61.5	91.5
	(0.2)	(169)	(2.1)	(3.1)	(0.08)	(352)	(16.9)	(8.8)
430 n*M*	11.7	236	14.5	75.4	11.8	2377	637*	47.9*
	(0.5)	(122)	(1.1)	(4.4)	(0.1)	(1899)	(257)	(6.9)
ANOVA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	$P < 0.05$	$P < 0.05$

[a] Transport of marker compounds (^{3}H-mannitol, HRP) and TEER during 5 hr of incubation at 37°C. Numbers are the means (SD) of three replications. All experiments were performed simultaneously using the same passage of cells. Mannitol data indicate the apparent permeability, calculated by linear regression. HRP data indicate the amount of cellular bound and of transported enzyme, respectively, the latter expressed as AUC of the nonlinear transport–time curve. Statistical analysis was done by one-way-ANOVA for the apical and basolateral treatments separately and testing for homogeneous groups on the basis of the 95% confidence intervals.

to lectin-induced changes in cellular morphology and metabolism, leading to an impaired barrier function of the gastrointestinal epithelium. Specifically, enhanced transcytosis of macromolecular drugs by bioadhesive carriers will require the identification of ligands which (i) are effectively internalized, (ii) are effectively transcytosed rather than being degraded or recycled, and (iii) do not affect the natural barrier function of the epithelium provided by tight intercellular junctions.

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REFERENCES

1. N. Sharon and H. Lis. *Lectins,* Chapman and Hall, London, 1989.
2. D. L. J. Freed and C. H. Buckley. Mucotractive effects of lectins. *Lancet* 18:585–586 (1978).
3. N. D. Noah, E. A. Bender, G. B. Reaidi, and R. J. Gilbert. Food poisoning from raw red kidney beans. *Br. Med. J.* 281:236–237 (1980).
4. M. S. Nachbar, J. D. Oppenheim, and J. O. Thomas. Lectins in the U.S. diet. Isolation and characterization of a lectin from the tomato (Lycopersicum esculentum). *J. Biol. Chem.* 5:2056–2061 (1981).
5. D. C. Kilpatrick, A. Pusztai, G. Grant, C. Graham, and S. W. B. Ewen. Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. *FEBS Lett.* 185:299–305 (1985).
6. C. M. Lehr, J. A. Bouwstra, W. Kok, A. B. J. Noach, A. G. de Boer, and H. E. Junginger. Bioadhesion by means of specific binding of tomato lectin. *Pharm. Res.* 9:547–553 (1992).
7. B. Naisbett and J. F. Woodley. Uptake of tomato lectin by the adult rat small intestine in vitro. *Biochem. Soc. Trans.* 19:879–880 (1989).
8. A. Pusztai. Transport of proteins through the membranes of the adult gastrointestinal tract—A potential for drug delivery? *Adv. Drug. Deliv. Rev.* 3:215–228 (1989).
9. J. Fogh, J. M. Fogh, and T. Orfeo. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J. Natl. Cancer Inst.* 59:221–226 (1977).
10. I. J. Hidalgo, T. J. Raub, and R. T. Borchardt. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelia. *Gastroenterology* 96:736–749 (1989).
11. A. R. Hilgers, R. A. Conradi, and P. S. Burton. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm. Res.* 7:902–910 (1990).
12. R. D. Schoenwald and H. S. Huang. Corneal penetration behavior of β-blocking agents. I. Physicochemical factors. *J. Pharm. Sci.* 72:1266–1272 (1983).
13. A. C. Maehly and B. Chance. In D. Glick, (ed.), *Methods of Biochemical Analysis,* Wiley–Interscience, New York, 1954, Vol. 1, pp. 357–398.
14. J. Wan, S. Persiano, and W.-C. Shen. Transcellular processing of disulfide and thioether-linked peroxidase-polylysine conjugates in cultured MDCK epithelial cells. *J. Cell. Physiol.* 145:9–15 (1990).
15. H. J.-P. Ryser, N. Morad, and W.-C. Shen. Heparin interactions with cultured cells: Possible role of fibronectin in uncoupling surface binding and endocytosis. *Cell Biol. Int. Rep.* 7:923–930 (1983).
16. G. J. Strous and J. Dekker. Mucin-type glycoproteins. *CRC Crit. Rev. Biochem. Mol. Biol.* 27:57–92 (1992).
17. M. Heyman, A.-M. Crain-Denoyelle, S. K. Nath, and J.-F. Desjeux. Quantification of protein transcytosis in the human colon carcinoma cell line Caco-2. *J. Cell. Physiol.* 143:391–395 (1990).
18. H. Hendriks, M. Kik, J. Koninkx, T. v. d. Ingh, and J. M. V. M. Mouwen. Binding of kidney bean (Phaseolus vulgaris) isolectins to differentiated human colon carcinoma Caco-2 cells and their effect on cellular metabolism. *Gut* 32:196–201 (1991).
19. J. Koninkx, H. Hendriks, J. van Rossum, T. v. d. Ingh, and J. M. V. M. Mouwen. Interaction of legume lectins with the cellular metabolism of differentiated Caco-2 cells. *Gastroenterology* 102:1516–1523 (1992).
20. M. Draaier, J. Koninkx, H. Hendriks, M. Kik, J. van Dijk, and J. Mouwen. Axtin cytoskeletal lesions in differentiated human colon carcinoma Caco-2 cells after exposure to soybean agglutinin. *Biol. Cell.* 65:29–35 (1989).
21. J. L. Madara, J. Stafford, D. Barenberg, and S. Carlson. Functional coupling of tight junctions and microfilaments in T84 monolayers. *Am. J. Physiol.* 254:G416–G423 (1988).