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The potential use of tomato lectin for oral drug delivery: 2. Mechanism of uptake in vitro

Barbara Naisbett *,[1], John Woodley

Drug Delivery Group, Department of Biological Sciences, Keele University, Keele ST5 5BG, UK

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Abstract

Tomato lectin (TL) is a non-toxic dietary glycoprotein of molecular mass 71 kDa. Its interaction with, and uptake by, the adult rat small intestine was investigated using an improved everted gut sac system, to evaluate its potential in oral drug delivery systems. Uptake of [^{125}I]TL was compared with two control molecules; ^{125}I-labelled polyvinylpyrrolidone (PVP), an inert polymer often used as a marker for fluid-phase endocytosis, and ^{125}I-labelled bovine serum albumin (BSA), a degradable protein of similar molecular mass to TL. Uptake of substrate associated with gut tissue or in the serosal space was calculated as ng per mg of gut sac protein. The rate of uptake of tomato lectin into gut sac tissue at 37°C was 27 ng/h per mg protein, but passage into the serosal space was much slower (1.7 ng/h per mg protein). The rate of uptake by the tissue was 11-times higher than BSA and 20-times higher than PVP, but transfer into the serosal space was only 4.3- and 5-times greater than BSA and PVP, respectively. This showed that the proportion of lectin transferred across the mucosa to the serosa was less than either control, and indicated accumulation of TL within the enterocytes. Incubation at 4°C and with metabolic inhibitors demonstrated that the mechanism of TL uptake by small intestinal gut sacs in culture was adsorptive endocytosis. Trichloroacetic acid solubility studies showed that TL was more resistant to enzyme degradation during incubation with gut sacs than BSA. The fact that TL was shown to adhere to the gut surface, showed increased uptake when compared with control macromolecules, PVP and BSA, and accumulated in the mucosal cells, may give it potential as a drug carrier for delivery to the gastrointestinal tract.

Key words: Tomato lectin; Oral drug delivery; Endocytosis; Bioadhesion; Drug carrier; Small intestine; Everted gut sac

1. Introduction

Whilst it is generally assumed that all ingested macromolecules undergo digestion prior to being absorbed, there is now much evidence to support the idea that macromolecules (particularly proteins) may penetrate the intestinal epithelial sur-

* Corresponding author.

[1] Present address: Leiden/Amsterdam Center for Drug Research, Division of Pharmaceutical Technology, Gorlaeus Laboratories, POB 9502, 2300 RA Leiden, The Netherlands. Tel: 31 71 274219; Fax: 31 71 274277.

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face, not in sufficient amounts to be of nutritional importance, but in quantities that may be biologically important (Vellenga et al., 1985; Walker, 1986; Bloch, 1988; Gardner, 1988; Heyman et al., 1989). This is important in the area of oral drug delivery, particularly considering the increasing number of (poly)peptides and proteins becoming available for use as drugs (Ferraiolo and Benet, 1985; Lee, 1990; Lee and Yamamoto, 1990), and in the area of oral immunisation (O'Hagan et al., 1987), where therapeutic methods for enhancing intact polypeptide absorption would be valuable for vaccine administration by the oral route (Gilligan and Li Wan Po, 1991).

Previously, we reported that a non-toxic macromolecule, a lectin from tomatoes, bound to enterocytes to a significant extent, and that this binding was sugar specific and mediated through brush border glycoproteins (Naisbett and Woodley, 1994). As well as having potential for increasing intestinal transit time, this adherence to the gut mucosal surface may also enhance the lectin's uptake into the enterocytes by endocytosis. This may be useful for drug delivery as endocytic capture of macromolecules has been used to try to achieve selective delivery of macromolecular drugs and drug carriers (Duncan, 1987). Furthermore, if a drug which is poorly absorbed in the intestine could be attached to a macromolecule such as tomato lectin, which binds avidly to the enterocytes in the gut, this may increase the drug's uptake and bioavailability.

Coupling to a macromolecule may also protect a degradable protein or peptide drug from intestinal peptidase action. For example, linking the peptide B-chain of insulin to polymers such as nVPMA (poly[*N*-vinypyrrolidone-co-maleic-anhydride]) and HPMA (*N*-[2-hydroxypropyl] methacrylamide) (Drobnik, 1989; Morgan et al., 1991) led to a decrease in B-chain degradation.

The work described in the current paper investigates the interaction of tomato lectin with the small intestinal surface in an improved everted gut sac system. The kinetics of this interaction were determined and the mechanism of the lectin's uptake was elucidated. Quantification and characterization of the uptake will be useful in evaluating the lectin's potential as a drug carrier and/or a bioadhesive in the gastrointestinal tract. The degradation of the lectin was also measured during uptake. The interaction of tomato lectin (TL) with the intestine was compared with two radiolabelled controls, polyvinylpyrrolidone (PVP), an inert polymer often used as a marker for fluid-phase endocytosis, and bovine serum albumin (BSA), a degradable protein of similar molecular mass to the lectin.

2. Materials and methods

2.1. Purification of tomato lectin

Tomato lectin was purified to homogeneity from the locular fluid of ripe tomatoes, in which it is the major protein, using ammonium sulphate precipitation and chromatofocusing, as described by Kilpatrick et al. (1983). Lectin activity was measured by the agglutination of untreated human erythrocytes (Kilpatrick and Yeoman, 1978). An 8-fold purification was obtained over the starting locular fluid. 5 mg of purified lectin was radiolabelled with 0.5 mCi of sodium [^{125}I]iodide (preparation IMS 30, Amersham International plc, U.K.), using Iodobeads (Pierce Chemical Co., IL, U.S.A.) as described by Kilpatrick et al. (1985). Following the labelling reaction, free [^{125}I]iodide was removed by extensive dialysis against 1% (w/v) sodium chloride. The amount of free [^{125}I]iodide in the reaction mixture and resultant preparations was estimated using paper electrophoresis (Whatman No. 1 filter paper, run in 0.05 M sodium barbitone buffer, pH 8.6, for 25 min at 400 mV, 10–15 mA). The specific activity of the ^{125}I-labelled lectin was approx. 30 μCi/mg. BSA was radiolabelled by the same method and [^{125}I]PVP was purchased from Amersham International plc, U.K.

2.2. Gut sac preparation and incubation

Everted intestinal sacs were prepared using the improved method developed by Bridges (1980). In this improved preparation, tissue culture medium is used as the incubation medium.

This ensures tissue viability and integrity for times up to 3 h. Adult male Wistar rats (250–300 g) were starved for 24 h, killed by cervical dislocation, and the small intestine excised and immediately placed in warm (37°C), oxygenated tissue culture medium 199 (TC199). The intestine was washed through with warm, oxygenated TC199, everted on a notched glass rod and placed in fresh medium. One end of the intestine was clamped and medium was introduced with a pipette into the intestine, which was then sealed with a second clamp. This large gut sac was divided into 12×1.5 cm sacs using braided silk sutures. Each sac was then separated, placed in a sterile Erlenmeyer flask (50 ml) containing 9.0 ml of pregassed (95% O_2: 5% CO_2) TC199 at 37°C, and preincubated for 5 min. Flasks were re-gassed and 1.0 ml of radiolabelled substrate, at a concentration of 20 μg/ml, was added to each flask at time zero. Flasks were stoppered with sterile silicon bungs and the sacs were incubated for times up to 2 h at 37°C, in an oscillating (70 strokes/min) water bath.

Sacs were removed at time points and blotted dry. The sacs were opened and the serosal fluid drained into a small flask. The volume of serosal fluid in each sac was measured using a syringe and any sacs that had leaked were discarded. The gut sacs were washed four times in ice-cold saline (0.85% w/v), blotted dry and digested individually in 25 ml of 1 M NaOH. Samples were taken for both protein estimation (method of Lowry et al. (1951), as modified by Peterson (1983)) and radioactive counting. Uptake of radiolabelled substrate by the sacs was plotted against time, and the rate of uptake into tissue and serosal space was calculated as ng of substrate/mg of gut sac protein per h. Endocytic indices were also calculated (see section 3).

2.3. Effect of substrate concentration

Gut sacs were cultured for 1 h in TC199 containing radiolabelled tomato lectin or PVP at concentrations ranging from 1 to 25 μg/ml. Uptakes were calculated as before and plotted against the concentration of the substrate in the medium.

2.4. Inhibitor studies

Gut sacs were cultured with 2 μg/ml [^{125}I]TL as before, but in the presence of metabolic inhibitors: sodium azide (10^{-3} M), an inhibitor of the electron transport chain; sodium fluoride (10^{-3} M), an inhibitor of glycolysis; or colchicine (5×10^{-5} M), a microtubule assembly inhibitor. [^{125}I]TL, [^{125}I]BSA and [^{125}I]PVP were also incubated with gut sacs for up to 2 h at low temperature (4°C). The specificity of lectin binding was determined by preincubating it with $[GlcNAc]_4$ at 1.4 μM (10-fold concentration excess of the lectin) prior to incubation with the gut sacs. In all of the above cases, the uptake of the substrates was calculated as described earlier and plotted against time.

2.5. Trichloroacetic acid precipitation

The degradation of the proteins was assessed by measuring the percentage of trichloroacetic acid-soluble fragments produced after incubation of [^{125}I]TL and [^{125}I]BSA with everted gut sacs. Trichloroacetic acid solubility of substrates in the external medium, serosal fluid and gut tissue was measured. Samples of incubation medium and serosal fluid were assayed directly, but tissue samples were neutralized with 1 M HCl prior to the precipitation procedure. 0.5 ml of 10 mg/ml BSA (as a carrier protein) was added to 1.0 ml of each sample and mixed well, followed by 1.0 ml of 20% (w/v) trichloroacetic acid. After thorough mixing, the tubes were centrifuged at $3500 \times g$ for 15 min at 4°C, and 1 ml of the supernatant was counted for radioactivity.

2.6. Integrity of the gut sacs

The 'leakiness' of the sacs was determined in each experiment by measuring the ratio of glucose concentration of the external medium to that of the serosal fluid. Viable enterocytes transport glucose against a concentration gradient, so in a non-leaking gut sac system, it should be possible to measure a glucose gradient between the external medium and the serosal fluid. Glucose was measured in gut sac incubation medium

and the serosal fluid using the GOD-PERID™ test kit (Boehringer Mannheim GmbH Diagnostica), following the manufacturer's instructions.

3. Results and discussion

3.1. Uptake of macromolecules at 37°C

It is important when studying pinocytic uptake to choose the correct units for expressing uptake. It is useful to be able to compare results from different tissues with different substrates. The concept of the endocytic index (E.I.) was proposed by Williams et al. (1975) to express uptake. The E.I. is defined as 'the volume of culture medium (μl) whose contained substrate is captured per mg of cell protein' and this allows direct comparison of rates of uptake in different experiments and by different cell types.

Although the units of E.I. are μl/mg tissue per h, this does not imply that an equivalent volume of fluid is ingested. If a substrate enters a cell entirely in the fluid phase, the volume taken up will be equivalent to the E.I. However, if a substrate enters mainly adsorbed to the membrane, the actual volume of liquid ingested will be far smaller than the calculated E.I. In the latter case it is usual to calculate uptake in terms of ng of substrate internalised. Because tomato lectin has been shown to bind specifically to the gut tissue, it seemed likely that uptake would be by adsorptive pinocytosis, and thus uptakes were calculated in ng/mg protein as well as an endocytic index. Uptakes of BSA and PVP were also calculated in this way to give direct comparisons with the lectin.

3.1.1. Tomato lectin

The binding and uptake of ^{125}I-labelled tomato lectin by everted gut sacs is shown in Fig. 1a. From the graph it can be seen that association of the lectin with gut tissue increases linearly with time (rate = 26 ng/h per mg protein). Uptake of radioactivity into the serosal fluid was also linear, but much slower, the rate being 1.7 ng/h per mg protein.

3.1.2. BSA and PVP

Fig. 1b shows the interaction of the two control macromolecules, [^{125}I]BSA and [^{125}I]PVP with gut sacs. The rate of uptake by the mucosa was 2.26 ng/h per mg protein for BSA and 1.26 ng/h per mg protein for PVP. Transfer into the serosal fluid occurred at 0.4 and 0.34 ng/h per mg protein for BSA and PVP, respectively. The values obtained for PVP uptake are comparable with those obtained by Bridges (1980) and Rowland and Woodley (1981).

The rate of uptake of lectin by the tissue was 11-times higher than BSA and 20-times higher

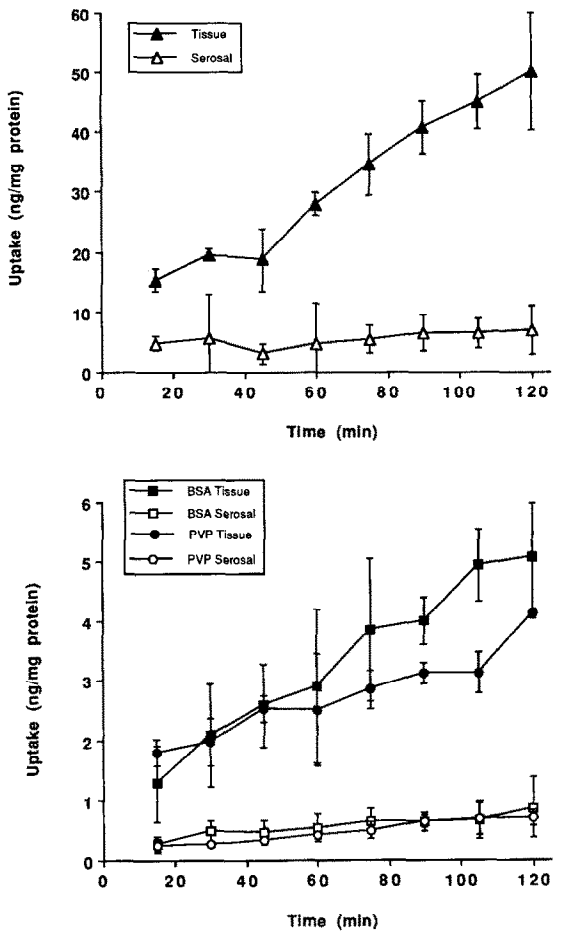

Fig. 1. (a) Uptake of [^{125}I]TL by gut sacs at 37°C. Each point represents the mean ± S.E. of 16 individual sacs. (b) Uptake of [^{125}I]BSA and [^{125}I]PVP by gut sacs at 37°C. Each point represents the mean ± S.E. of 16 individual sacs.

Table 1
Rates of uptake of macromolecules by gut sacs (ng/h per mg protein)

	Tissue	Serosal	Tissue/serosal ratio
Lectin	26.00	1.70	15.3
BSA	2.26	0.40	5.7
PVP	1.26	0.34	3.7

than PVP, but transfer into the serosal fluid was only 4.3- and 5-times greater than BSA and PVP, respectively. This showed that the proportion of lectin transferred across the mucosa into the serosal fluid was less than either control (although the overall amount of lectin transfer was greater), and indicated accumulation of lectin within the enterocytes. This can be seen more clearly in Table 1, where the tissue to serosal ratio is an indication of the rate of transfer from tissue into the serosal fluid.

3.2. Determination of mechanism of uptake

During endocytosis, substrates may be captured in two possible ways, either in solution (fluid-phase endocytosis) or attached to the invaginating plasma membrane by specific receptors or non-specific adsorption. Substrates with no affinity for the enterocyte membrane are taken up solely in solution, whereas substrates with some degree of membrane affinity can be taken up bound to the membrane, or by a combination of both fluid and adsorptive modes.

During fluid-phase endocytosis, an increase in substrate concentration in the extracellular medium results in uptake (expressed in ng) increasing linearly with concentration. A substrate entering in the adsorptive mode also does so in a concentration-dependent manner, but as the concentration increases, the sites to which the substrate binds become saturated, and uptake will level off. This uptake is therefore dependent on the number, affinity and function of the cell surface binding sites.

Metabolic and cytoskeletal inhibitors have little or no effect on the binding component of substrate association with the enterocytes, but they block uptake by fluid-phase endocytosis, as energy and cytoskeletal elements are needed for the movement and fusion of membranes and vesicles. Low temperature also has the same effect as metabolic inhibitors with substrates binding to cell surfaces at 0°C without internalisation as there is no movement of membranes at this temperature due to a change in membrane fluidity.

It was possible therefore to determine the nature of the mechanism of tomato lectin uptake by incubating everted gut sacs with various metabolic inhibitors, cytoskeletal inhibitors, low temperature and at different substrate concentrations.

3.2.1. Effect of substrate concentration

Gut sacs were cultured for 1 h with ^{125}I-labelled lectin or [^{125}I]PVP at concentrations from 1 to 25 μg/ml. The rate of uptake of ^{125}I-labelled substrates was plotted against the concentration of substrate.

The effect of concentration on tomato lectin uptake is shown in Fig. 2a. The rate of uptake by the tissue and the rate of appearance of the lectin in the serosal fluid increased with substrate concentration up to 10–15 μg/ml, although neither rate was linear. At higher concentrations, saturation occurred and there was a slight reduction in rate. The non-linearity of uptake and saturation at higher substrate concentrations indicated that adsorptive pinocytosis was the mechanism by which tomato lectin entered the enterocytes.

Fig. 2b shows the uptake of [^{125}I]PVP at increasing substrate concentrations is linear and unsaturable. This result was consistent with a fluid-phase mode of endocytic uptake in agreement with other authors (Bridges, 1980; Pratten et al., 1980; Rowland and Woodley, 1981). It is also interesting to note that at the saturation concentration for TL (15 μg/ml), uptake of tomato lectin was 40-times greater than PVP at the same concentration.

3.2.2. Effect of inhibitors

Endocytosis is dependent on metabolic energy and inhibitors of glycolytic and oxidative metabolism will reduce endocytic uptake in many cell types without affecting free diffusion and facilitated diffusion mechanisms. In this study,

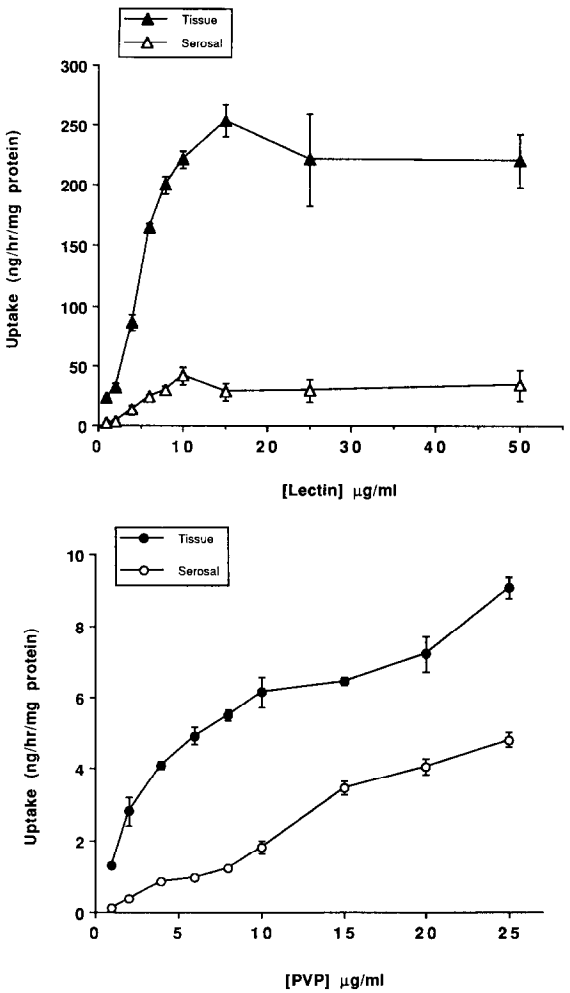

Fig. 2. (a) Effect of lectin concentration on uptake by gut sacs. Each point represents the mean ± S.E. of 16 individual sacs. (b) Effect of PVP concentration on uptake by gut sacs. Each point represents the mean ± S.E. of 16 individual sacs.

inhibitors of cellular glycolysis, oxidative phosphorylation and microtubule assembly were employed to determine the energy requirements for tomato lectin absorption in the adult rat small intestine. Table 2 summarizes the effects of the inhibitors used in this study, as described in section 2.

Colchicine was the most effective inhibitor used with respect to tissue uptake, inhibiting by 72% (rate = 7.29 ng/h per mg protein) at a concentration of 5×10^{-5} M, which was much lower than

Table 2
Effect of inhibitors on rates of uptake (ng/h per mg protein)

Inhibitor	Tissue		Serosal	
	Rate	% inhibition	Rate	% inhibition
NaN_3 (10^{-3} M)	7.05	73	0.49	71
NaF (10^{-3} M)	12.01	54	1.00	41
Colchicine (5×10^{-5} M)	7.29	72	2.16	27 (increase)
$[GlcNAc]_4$	6.32	76	0.71	58

that used with fluoride or azide. However, the rate of serosal accumulation was increased compared with the control, the rate being 2.16 ng/h per mg protein. Uninhibited serosal uptake of lectin was approx. 1.7 ng/h per mg protein. Table 3 shows the medium to serosal glucose gradients obtained when the sacs were incubated with lectin and the various inhibitors. It can be seen that addition of colchicine destroyed the glucose gradient between the external medium and the serosal space. The observations that colchicine increased serosal transfer of TL in the absence of increased tissue-associated radioactivity and also abolished the glucose gradient indicated colchicine-induced toxicity, making the sacs 'leaky'. The concentrations of colchicine used in this study had previously been shown to inhibit gut sac uptake (Bridges, 1980; Rowland and Woodley, 1981), but the effect on glucose uptake had not been investigated.

Colchicine binds to tubulin in cells, leading to tubule depolymerization. As microtubules are in-

Table 3
Effect of inhibitors on observed glucose gradients

Inhibitor	Time (min)	Medium:serosal
Control	15	1:1.4
	120	1:3.3
NaN_3	15	1:1.1
	120	1:3.1
NaF	15	1:1.1
	120	1:3.0
Colchicine	15	1:1.0
	120	1:1.0
$[GlcNAc]_4$	15	1:1.3
	120	1:3.5

volved in the overall organization of the cytoskeleton and the movement of pinocytic vesicles, the presence of colchicine inhibits vesicle movement and reduces pinocytic uptake. As colchicine interferes with the cytoskeleton, it is also possible that it may cause disruption of the tight junctions between the enterocytes. Madara (1989) has suggested that the zonula occludens may be manipulated by the cytoskeleton, and thus any pertubation of the cytoskeleton may permeabilize the tight junctions to large molecules such as tomato lectin. This would explain the leakiness of the sacs and the influx of lectin into the serosal space.

Sodium azide and sodium fluoride inhibited serosal transfer by 71 and 41%, respectively. A similar reduction in tissue uptake was also observed (73 and 54%, respectively). Residual tissue-associated activity was thought to be predominantly lectin which has bound to the surface of the mucosa, but, because of the presence of metabolic inhibitors, could not be internalised (or the rate of internalisation was much reduced). From this study, however, bound and internalised lectin could not be distinguished.

100% inhibition was never achieved with the inhibitors, although the concentrations used were relatively high. Using the same concentration of metabolic inhibitors, Bridges (1980) obtained a similar result, with maximum inhibitions around 50%. Energy for uptake in the intestine is derived from more than one source, and it has been claimed (Windmueller and Spaeth, 1980) that, in vivo, the small intestinal mucosa derives much of its energy from glutamine. Glutamine (present in TC199 medium at 0.1 mg/ml) can enter the citric acid cycle and be metabolized, producing energy, and this is not affected by the addition of sodium fluoride. In the presence of azide, it is likely that there is a switch to anaerobic metabolism, which would produce enough energy to maintain some uptake. The fact that two different metabolic inhibitors significantly reduce the rate of uptake was strongly indicative that the mechanism of lectin uptake was endocytosis.

After preincubation of tomato lectin with $[GlcNAc]_4$, uptake of lectin into the tissue and subsequent serosal transfer was reduced by 76%

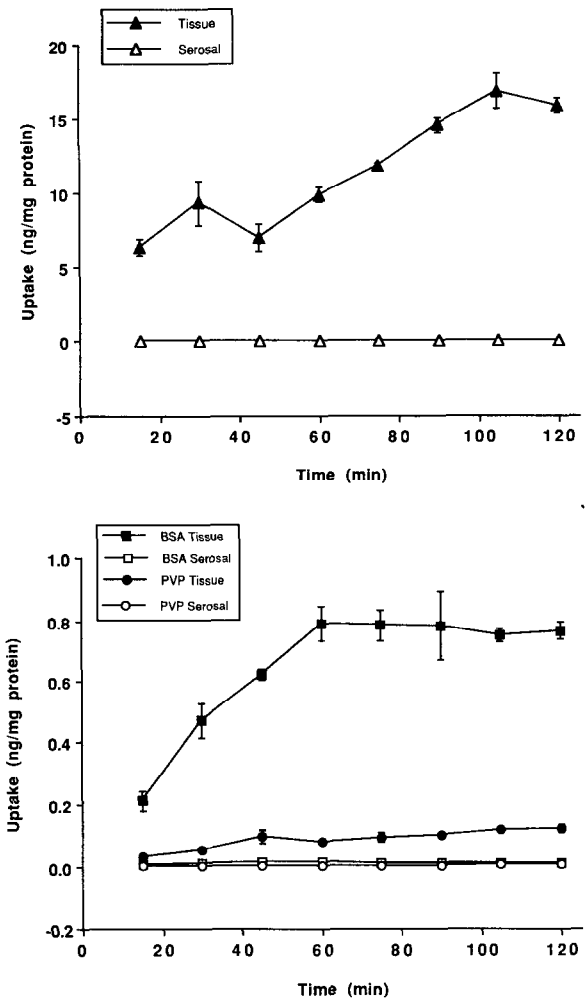

Fig. 3. (a) Uptake of [^{125}I]TL by gut sacs at 4°C. Each point represents the mean ± S.E. of 16 individual sacs. (b) Uptake of [^{125}I]BSA and [^{125}I]PVP at 4°C. Each point represents the mean ± S.E. of 16 individual sacs.

(rate = 6.32 ng/h per mg protein) and 58% (rate = 0.71 ng/h per mg protein), respectively. These data showed that lectin binding to the gut mucosa was specific.

3.2.3. *Uptake of macromolecules at 4°C*

The binding and uptake of [^{125}I]TL, [^{125}I]BSA and [^{125}I]PVP with everted gut sacs at 4°C is shown in Fig. 3a and b. Fig. 3a shows that interaction of tomato lectin with the gut mucosa was reduced by 78% compared with its uptake at

37°C (rate = 5.7 ng/h per mg protein), but not totally abolished. This showed that the majority of the lectin activity found in the tissue at 37°C represented internalised lectin, with a surface binding component of approx. 20%. Uptake into the serosal space was negligible at 4°C. As a substrate taken up solely by fluid-phase pinocytosis would show no tissue-associated activity at 4°C, the residual tissue activity, together with competing sugar results, showed that the mechanism of lectin uptake was by specific adsorptive endocytosis.

BSA interaction with the gut mucosa was reduced by 82% to 0.4 ng/h per mg protein at 4°C. The persistence of some 18% tissue-associated activity at 4°C indicated uptake by an adsorptive mechanism. Virtually no radioactivity could be detected in the serosal space. Experiments measuring the effect of BSA concentration on uptake (data not shown) suggest that the mode of uptake of BSA is one of non-specific adsorptive endocytosis.

Very little [^{125}I]PVP was found in the gut tissue at 4°C, indicating little or no binding of PVP to the enterocytes, the results being consistent with a fluid-phase endocytic mode of uptake for PVP.

3.3. Degradation of macromolecules

The degradation of tomato lectin and BSA by intestinal peptidases will produce peptide fragments which will be soluble in the presence of trichloroacetic acid. Thus, trichloroacetic acid precipitation is a simple method to obtain a measure of the degradation of a radiolabelled protein. The percentage of acid-soluble fragments produced by incubation of [^{125}I]TL and [^{125}I]BSA with everted gut sacs was measured as described in section 2. The results are listed in Table 4.

These results show clearly that lectin in the medium is more resistant to proteolysis during the incubation with the sacs (3.9% solubilty compared with 10.0% for BSA), where the breakdown is a result of the activity of peptidases on the enterocyte surface. The lectin was also more resistant within the tissue, as only 6.3% solubility was obtained after 2 h incubation, compared with 23.2% solubility for BSA.

Table 4
Trichloroacetic acid solubility after incubation with gut sacs (%)

	0 min	15 min	60 min	120 min
Lectin				
Medium	< 1	1.4	2.5	3.9
Tissue	–	2.1	2.6	6.3
Serosal	–	48.7	50.8	50.1
BSA				
Medium	< 1	1.7	5.9	10.0
Tissue	–	2.8	10.1	23.2
Serosal	–	47.1	53.2	51.3

The percentage of soluble fragments in the serosal fluid was similar for both lectin and BSA (50%) at all time points measured. The percentage of trichloroacetic acid soluble fragments in the serosal fluid did not increase during incubation, indicating that, for both lectin and BSA, a proportion of the serosal-associated radioactivity was present as high molecular mass fragments. This suggested that partially digested fragments of tomato lectin and BSA could be transferred from the tissue to the serosal fluid.

As tomato lectin exhibited greater resistance to degradation in both the external medium and tissue than BSA, it was unexpected to find similar trichloroacetic acid solubilities in the serosal fluid for both molecules. However, the method of trichloroacetic acid precipitation gave no information on the nature or size of the soluble and insoluble fragments produced, and it was possible that the trichloroacetic acid precipitable fraction of TL in the serosal fluid contained fragments that were much larger than those produced by digestion of BSA.

In this paper, experiments were designed to investigate the interaction of tomato lectin with the adult rat small intestine in vitro, to determine the suitability of the lectin as a macromolecular carrier (or bioadhesive) for oral drug delivery systems.

The tomato lectin was shown to be taken up by enterocytes via specific adsorptive endocytosis and this uptake was significantly greater than that of

Table 5
Endocytic indices of macromolecules taken up by the adult rat small intestine

Substrate	Concentration (μg/ml)	Endocytic index (μl/h per mg protein)		Reference
		Tissue	Serosal	
Tomato lectin	2.0	13.00	0.85	This study
Polyvinylpyrrolidone	2.0	0.63	0.17	This study
Polyvinylpyrrolidone	2.0	0.74	0.12	Bridges et al. (1987)
Bovine serum albumin	2.0	1.13	0.20	This study
(*N*-[2-Hydroxypropyl]methacrylamide	?	0.61	0.33	Cartlidge et al. (1987)
Horseradish peroxidase	?	1.13	0.39	Beahon and Woodley (1984)

two control macromolecules, BSA and PVP. The rate of uptake of tomato lectin by gut sacs, in terms of an endocytic index, can be compared with other substrates (Table 5). The higher uptake for tomato lectin, compared with the other substrates was a consequence of adsorptive pinocytosis, as this mechanism is known to enhance the rate of substrate capture (Pratten et al., 1980). The studies with competing sugar showed that this adsorption of the lectin was specific. Because of this high rate of endocytic capture, the coupling of tomato lectin to a drug may be used to increase the drug's uptake in the gastrointestinal tract.

The attachment of drugs to macromolecular carriers automatically limits cellular capture of drug-carrier to the endocytic route (Duncan, 1987), and once internalized, a carrier must undergo intracellular processing to release the drug in an active form. Efficient endocytosis (and post-endocytic processing) is therefore critical for drug delivery by this method (Ryser et al., 1990). The most efficient method of internalization is by receptor-mediated endocytosis. However, Ryser et al. (1990) have suggested that adsorptive endocytosis can also be a highly efficient process when the binding sites are numerous and of high affinity.

Some lectins have been investigated as potential drug carriers, and enzymes (dextranase, asparaginase and trypsin) and cytotoxic agents (daunomycin) have been covalently coupled to Concanavalin A (Shier, 1979). These reports suggest that it would be possible to covalently couple lectins, such as tomato lectin, to a low molecular mass drug or a protein/peptide drug and oral bioavailability may be improved. The coupling may protect the drug from enzymic inactivation or enable the drug to be released in the correct intracellular compartment. The covalent coupling to lectin will also direct a non-absorbable drug towards an endocytic route of capture, the rate of which may be increased by specific bioadhesion.

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