J. Membrane Biol. 97, 259–266 (1987)

The Journal of
Membrane Biology

The Na^+-Independent D-Glucose Transporter in the Enterocyte Basolateral Membrane: Orientation and Cytochalasin B Binding Characteristics

D.D. Maenz and C.I. Cheeseman
Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7 Canada

Summary. Phloridzin-insensitive, Na^+-independent D-glucose uptake into isolated small intestinal epithelial cells was shown to be only partially inhibited by trypsin treatment (maximum 20%). In contrast, chymotrypsin almost completely abolished hexose transport. Basolateral membrane vesicles prepared from rat small intestine by a Percoll® gradient procedure showed almost identical susceptibility to treatment by these proteolytic enzymes, indicating that the vesicles are predominantly oriented outside-out. These vesicles with a known orientation were employed to investigate the kinetics of transport in both directions across the membrane. Uptake data (i.e. movement into the cell) showed a K_t of 48 mM and a V_{max} of 1.14 nmol glucose/mg membrane protein/sec. Efflux data (exit from the cell) showed a lower K_t of 23 mM and a V_{max} of 0.20 nmol glucose/mg protein/sec. D-glucose uptake into these vesicles was found to be sodium independent and could be inhibited by cytochalasin B. The K_i for cytochalasin B as an inhibitor of glucose transport was 0.11 μM and the K_D for binding to the carrier was 0.08 μM. D-glucose-sensitive binding of cytochalasin B to the membrane preparation was maximized with L- and D-glucose concentrations of 1.25 M. Scatchard plots of the binding data indicated that these membranes have a binding site density of 8.3 pmol/mg membrane protein. These results indicate that the Na^+-independent glucose transporter in the intestinal basolateral membrane is functionally and chemically asymmetric. There is an outward-facing chymotrypsin-sensitive site, and the K_t for efflux from the cell is smaller than that for entry. These characteristics would tend to favor movement of glucose from the cell towards the bloodstream.

Key Words Small intestine · glucose transport · cytochalasin B binding · membrane vesicles

Introduction

The absorption of D-glucose across the epithelia of the small intestine is a two-stage process involving Na-dependent secondary active transport across the brush border and a Na-independent carrier-mediated diffusion mechanism located in the basolateral membrane. Considerable attention has been directed toward the Na-dependent carrier, while much less is known about the exit step in the net process of glucose absorption. Na-independent glucose transport by the intestine is sensitive to inhibition by cytochalasin B [9], suggesting a similarity to the transporter found in nonepithelial cell types such as the erythrocyte and the adipocyte. Preliminary evidence using isolated cells [9] and purified basolateral membrane vesicles [19] indicates that the K_t for Na-independent glucose transport in the intestine is in the range of 35 to 55 mM.

The detailed characteristics of Na-independent D-glucose transport in several nonepithelial cell types have been determined. In the red cell, the transporter is known to be functionally asymmetric, with a K_t for uptake into the cell of 1 to 6 mM and a four- to 15-fold higher value for the K_t of efflux [8, 13]. Further, it is thought that the red cell glucose transporter is oriented with a trypsin-sensitive site exposed on the inner (cytoplasmic) surface and a chymotrypsin-sensitive site exposed on the outer surface of the membrane [1, 3, 15]. Cytochalasin B is a potent inhibitor of D-glucose transport in the red cell, with a K_i of 0.1 μM [7]. The K_D for D-glucose-inhibitable ^{3}H-cytochalasin B binding is similar to the K_i for inhibition of transport and the measure of ^{3}H-cytochalasin B binding has proven to be useful for characterizing and quantifying D-glucose transport sites in red cells [11], adipocytes [17] and muscle cells [10].

Recent observations have shown that Na-independent glucose transport across the intestine can be induced by hyperglycemia or streptozotocin-induced diabetes [4, 12]. These studies implicate the basolateral membrane as an important site for the regulation of intestinal glucose transport. However, the mechanism of induction, be it an increase in the number of functional transporters or an increase in the glucose translocation rate of individual transporters, is not known.

Our objectives were to use isolated epithelial cells and purified basolateral membrane vesicles obtained from rat small intestine to (1) determine the

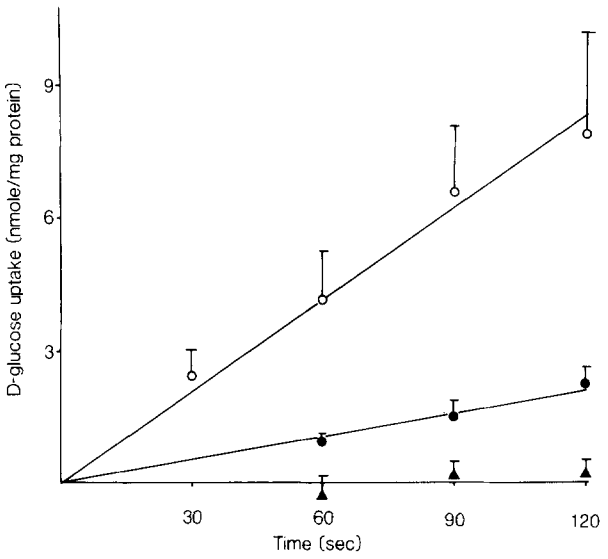

Fig. 1. D-glucose uptake into isolated intestinal epithelial cells at 60, 90 and 120 sec. The incubation medium contained 1 mM L- and D-glucose and [^{3}H] L-glucose and [^{14}C] D-glucose. Uptakes were performed at 37°C and the solutions were shaken at 90 oscillations per min. The points represent the mean values of net D-glucose uptake from four experiments. Open circles: control total uptake; filled circles, uptake in the presence of 1 mM phloridzin; filled triangles, uptake in the presence of 1 mM phloridzin + 100 μM cytochalasin B. Error bars indicate SEM

orientation of the D-glucose transporter in the basolateral membrane, (2) determine the kinetic parameters for D-glucose transport in both directions across the basolateral membrane, and (3) characterize D-glucose-inhibitable ^{3}H-cytochalasin B to the membrane. These experiments allow a comparison to be made between Na-independent D-glucose transport and cytochalasin B binding characteristics in nonepithelial cell types and the enterocyte, and they establish an assay procedure for the quantification of transporter in the basolateral membrane of the small intestine.

Materials and Methods

Preparation of Isolated Cells

Cells were isolated from the whole jejunum and ileum using the technique of Watford et al. [18]. These were suspended in Krebs bicarbonate buffer containing 1 g % albumin and 5 mM glutamine as substrate. For the measurement of D-glucose uptake, all incubations were performed in the presence of L-glucose, which was used to estimate extracellular space and passive permeation. 1.0 mM phloridzin was employed to block uptake across the brush-border membrane and cytochalasin B (100 μM) to block uptake of glucose across the basolateral membrane. Enzyme pretreatment experiments involved incubating 1 ml of cell suspension with 2 ml of enzyme in the normal incubation medium at 37°C; phloridzin was added 60 sec before determining uptake of glucose. Glucose uptake was initiated by adding 200 μl of 15 mM L- and D-glucose and ^{14}C D-glucose and ^{3}H L-glucose. Uptakes were terminated by pouring the incubation medium containing the cells into 10 ml of ice-cold Krebs bicarbonate saline and centrifuging for 3 min at 1200 rpm. The supernatant was removed, the cells resuspended in 1 ml wash solution (Krebs saline at 4°C) and an aliquot removed for protein determination before pelleting the cells. After removing the supernatant, 300 μl of 0.05 N nitric acid was added to the cells, which were left for 1 hr before placing the plastic tube in a scintillation vial for liquid scintillation counting.

At all stages of preparation and incubation, plastic containers were employed to minimize cell clumping.

Preparation of Basolateral Membrane Vesicles

Basolateral membrane vesicles (BLV) were prepared from rat small intestinal mucosal scrapings by differential centrifugation and final purification on a Percoll® gradient as described previously [12]. The final BLV pellet showed a 17-fold enrichment in the specific activity of Na^+,K^+-ATPase with little contamination by brush-border or microsomal fractions [12].

Transport Across BLV

For Na-independent D-glucose transport assays, the final BLV pellet was resuspended in vesicle resuspension solution (125 mM KSCN, 2 mM Tris-HCl, pH 7.4) at a concentration of 8.4 mg protein/ml.

Initial rates of D-glucose uptake across the vesicles were determined using 3-sec incubation periods. A 10-μl drop of BLV and a 10-μl drop of uptake solution (125 mM KSCN, 2 mM Tris-HCl, pH 7.4, D-glucose + ^{3}H-D-glucose) were placed in close proximity on the bottom of a polycarbonate tube. Uptake was initiated by vortexing the tube, and at the conclusion of the incubation period, 1.125 ml of ice-cold stop solution (125 mM NaCl, 2 mM Tris-HCl, pH 7.4, 0.1 mM $HgCl_2$) was injected into the mixture and 1.0 ml of the diluted mixture was transferred to a cellulose acetate filter (0.45 μm pore size). The filter was washed with 5 ml ice-cold stop solution, then placed in a scintillation vial for liquid scintillation counting. Glucose actually held by the vesicles was calculated by correcting for ^{3}H-D-glucose retained on the filter in the absence of vesicles.

To measure D-glucose efflux, BLV's were preloaded with D-glucose and ^{3}H-D-glucose during an overnight incubation at 0°C. The final concentration of preloaded vesicles was 8.4 mg protein/ml. A zero-time sample was taken by placing a 10-μl drop of preloaded BLV on the side of a plastic culture tube above the fluid line of 500 μl of ice-cold stop solution. The tube was then vortexed, the contents immediately transferred to a cellulose acetate filter and the glucose content of the vesicles determined as described for uptake. Efflux samples were determined by placing 10 μl of preloaded BLV above 500 μl of room temperature vesicle resuspension solution. The tube was then vortexed for the required period of efflux and BLV glucose content was determined.

^{3}H-Cytochalasin B Binding to BLV

Cytochalasin B binding assays were performed using a modification of the technique of Cushman and Wardzala [5] as applied to

Table 1. Effect of trypsin and chymotrypsin pretreatment on phloridzin-insensitive D-glucose uptake into isolated enterocytes[a]

Control	Trypsin				Chymotrypsin			
	Enzyme activity (U/ml)				Enzyme activity (U/ml)			
	40,000	80,000	120,000	160,000	200	400	600	800
3.60 ± 0.44	2.87 ± 0.76	2.91 ± 0.55	3.22 ± 0.60	2.92 ± 0.37	2.15 ± 0.17*	0.79 ± 0.49**	0.88 ± 0.34**	0.58 ± 0.42***
% 100	80	81	89	81	60	22	24	16

[a] Isolated enterocytes were incubated with trypsin or chymotrypsin for 5 min before measuring D-glucose uptake in the presence of 1 mM phloridzin. Uptake (nmol/mg protein) was measured using a 2-min incubation period and L-glucose was employed to correct for extracellular space and passive permeation. Values are the means of four experiments ± SEM. *t*-tests showed means were significantly different at $P <$ *0.05, **0.01 or ***0.001.

adipocytes. BLV's were resuspended in 250 mM mannitol and 2 mM Tris-HCl, pH 7.4, at a concentration of 4.0 mg BLV protein/ml. For each determination of D-glucose-inhibitable ^{3}H-cytochalasin B binding, matching assays were carried out in the presence of L-glucose and D-glucose. A 10-min glucose preincubation period was initiated by transferring (i) 200 μl BLV to 500 μl L-glucose solution (L-glucose and cytochalasin E as required, 2 mM Tris-HCl, pH 7.4), and (ii) 200 μl BLV to 500 μl D-glucose solution (D-glucose and cytochalasin E, 2 mM Tris-HCl, pH 7.4). After the preincubation period, 50 μl of ^{3}H-cytochalasin B solution (^{3}H-cytochalasin B and ^{14}C-urea as required [^{3}H/^{14}C cpm ratio = 2 : 1], 2 mM Tris-HCl, pH 7.4) were added to each of the L-glucose and D-glucose incubation mixtures. Triplicate samples of 25 μl were removed from each mixture and added directly to scintillation vials to determine total ^{3}H and ^{14}C dpm. Aliquots of 3 × 200 μl were then transferred from each mixture to a total of six airfuge tubes and the tubes were loaded into a six-well rotor and centrifuged for 30 min at 30 PSI in a Beckman airfuge (Beckman Instruments). For each airfuge tube, duplicate 25-μl samples of supernatant were transferred to scintillation vials and the remaining supernatant was removed by aspiration. Fifty μl of 1.0 M NaOH was added to dissolve the pellet and the tube was placed in a drying oven for 1 hr. The dissolved pellet was neutralized with HCl and the airfuge tube and contents were placed in a scintillation vial. ^{3}H and ^{14}C dpm were determined by liquid scintillation counting for total, supernatant and pellet samples. pmol of cytochalasin B bound to BLV was calculated after correcting for unbound ^{3}H-cytochalasin associated with the pellet by using ^{14}C-urea as a nonbinding aqueous space marker.

Materials

Protein was determined using the Sigma assay kit following the method of Bradford [2].

^{3}H-D-glucose, ^{14}C-D-glucose, ^{3}H-L-glucose, ^{3}H-cytochalasin B and ^{14}C-urea were purchased from Amersham. Cytochalasin B, cytochalasin E, D-glucose, L-glucose, trypsin and chymotrypsin were purchased from Sigma Chemical Company.

Results

D-Glucose Uptake by Isolated Cells

Figure 1 shows the uptake of D-glucose into the isolated cells over a period of 2 min after correcting for extracellular space and diffusion. One mM phloridzin was able to inhibit a sizeable component of the uptake, but the remaining uptake could be abolished by 100 μM cytochalasin B. This indicates that the phloridzin-insensitive uptake represents transport across the basolateral membrane (*see also* vesicle data below).

Pretreatment of the cells for 5 min with varying concentrations of trypsin and chymotrypsin had markedly different effects. Trypsin may have caused a marginal (10 to 20%) inhibition of phloridzin-insensitive D-glucose uptake, although this effect was not statistically significant at any one concentration. In contrast, chymotrypsin at only 200 units/ml reduced uptake by 40%, and at 400 to 800 units/ml transport was reduced to 20% of control values (Table 1).

D-Glucose Transport Across Purified Basolateral Membrane Vesicles

Previous work in this laboratory established conditions for measuring the initial rate of Na-independent D-glucose uptake across purified BLV [12]. D-glucose was shown to equilibrate into an osmotically active intravesicular space with no significant binding to the membrane. Incubation periods of 3 sec or less gave consistent estimations of the initial rate of D-glucose uptake across BLV.

Figure 2 shows the time-course for Na-independent D-glucose efflux from preloaded BLV. In subsequent experiments a 10-sec incubation period was used to measure initial rates of D-glucose efflux.

Sidedness of BLV Preparation

We designed experiments to determine the sidedness of our BLV preparation by measuring the susceptibility of glucose uptake to inactivation by trypsin versus chymotrypsin. Preliminary experiments established a range of enzyme concentrations which

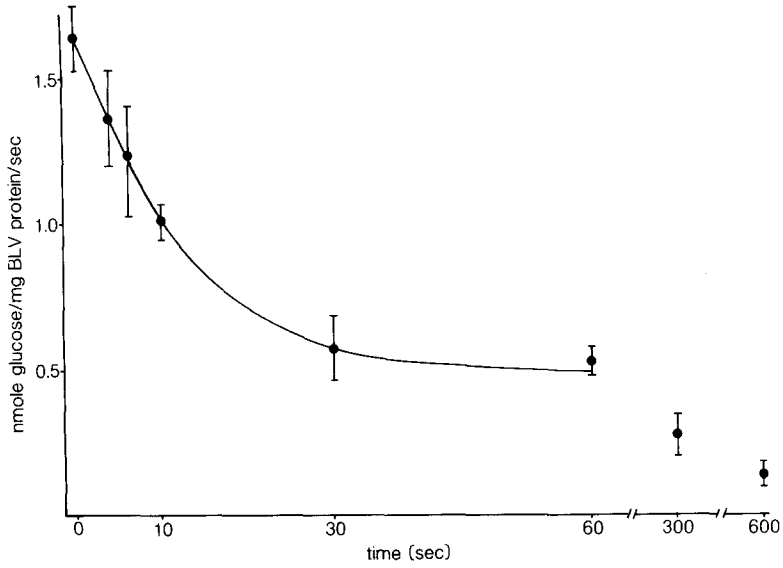

Fig. 2. Time-course of D-glucose efflux from preloaded BLV. BLV were prepared, preloaded with D-glucose + ^{3}H-D-glucose (final concentration = 2.0 mM), and efflux was performed as described in Materials and Methods. The points represent the mean ± standard error of triplicate determinations at each of the indicated periods of efflux

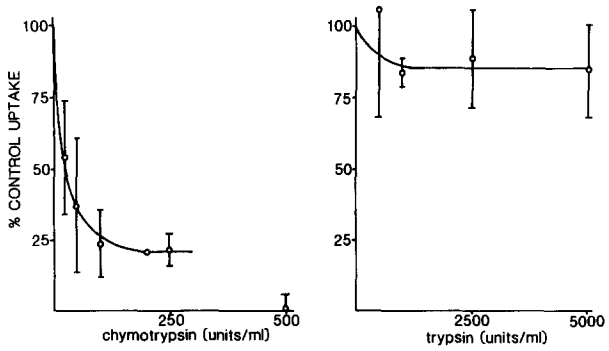

Fig. 3. Effect of chymotryptic and tryptic digestion of basolateral membrane vesicles on the initial rate of carrier-mediated D-glucose uptake. For a given experiment, BLV's were preincubated for 5 min with control, cytochalasin B or enzyme-containing solutions. The final concentrations in the various digestion mixtures were as follows: BLV, 8.4 mg protein/ml; cytochalasin B, 25 μM; trypsin and chymotrypsin, as indicated. Triplicate D-glucose uptakes were performed as described in Materials and Methods on each digestion mixture using a final concentration of 2.0 mM D-glucose + ^{3}H-D-glucose. Carrier-mediated uptake was calculated by correcting for the diffusion component of total uptake as determined in the presence of excess cytochalasin B and the results were expressed as a percentage of the initial rate across control vesicles. The data points indicate the means and standard error obtained from four preparations of BLV

caused a specific inactivation of transport after a 5-min digestion period. Longer incubation periods (>30 min) or excessive concentrations of enzyme resulted in a nonspecific deterioration of the vesicles.

Figure 3 shows the effects of varying concentrations of trypsin and chymotrypsin on the initial rate of transport-mediated D-glucose uptake when expressed as a percentage of uptake across control vesicles. Chymotryptic digestion (100 to 250 units/ml) caused an 80% inactivation of transport, while tryptic digestion resulted in an average loss of 15% of D-glucose transport activity in the four batches of BLV tested.

Kinetic Parameters of D-Glucose Transport Across BLV

The initial rate of D-glucose uptake across BLV is composed of transport plus noncarrier-mediated diffusion. Figure 4 shows a Hofstee transformation of initial rate data illustrating the two components of total uptake. At D-glucose concentrations over 25 mM, transport-mediated uptake is saturated. Preincubating the vesicles with 25 μM cytochalasin B abolished transport and allowed for an estimate of noncarrier-mediated diffusion. After correcting for diffusion, the kinetic parameters of Na-independent D-glucose transport were determined using the Hofstee transformation. This plot gives a V_{max} of 0.87 ± 0.06 nmol glucose/mg BLV protein/sec and a K_t of 35 ± 3 mM D-glucose.

Table 2 summarizes the data obtained from four separate determinations of K_t and V_{max} for transport-mediated D-glucose uptake and efflux across BLV. These experiments indicate an asymmetry in D-glucose transport with a twofold higher K_t and a fivefold higher V_{max} for flux in the uptake direction.

K_i for Cytochalasin B Inhibition of Transport

The previous experiments indicate that cytochalasin B is an effective inhibitor of D-glucose transport

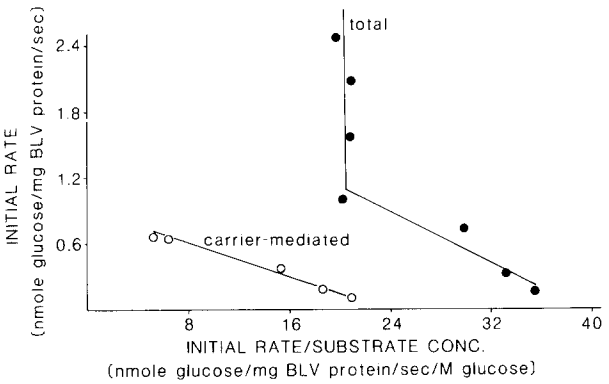

Fig. 4. Hofstee plot of initial rates of D-glucose uptake across BLV. A series of uptake media were prepared containing D-glucose + ^{3}H-D-glucose to give final concentrations, after mixing with the vesicles, of 125, 100, 75, 50, 25, 10, 5 mM D-glucose. Mannitol was added to the uptake media such that in all cases the final concentration of D-glucose + mannitol equaled 125 mM. Triplicate initial rate determinations were made for each uptake media across control BLV and BLV pretreated for 5 min with 25 μM cytochalasin B. The filled circles represent the Hofstee transformation of the mean values obtained across control BLV and the open circles represent the carrier-mediated component of uptake after correcting for unsaturable diffusion as determined in the presence of excess cytochalasin B

Table 2. Kinetic parameters of carrier-mediated D-glucose transport across BLV

Expt. #	Uptake[a]		Expt. #	Efflux[b]	
	K_t (mM)	V_{max} (nmol/mg/sec)		K_t (mM)	V_{max} (nmol/mg/sec)
1	36	870	1	28	197
2	54	1232	2	21	200
3	56	1429	3	19	185
4	46	1011	4	22	231
$X \pm$ SE	48 ± 5	1138 ± 141	$X \pm$ SE	23 ± 2	203 ± 11

[a] Kinetics of carrier-mediated D-glucose uptake across BLV were determined on each of four batches of vesicles as described in Fig. 4.

[b] Initial rates of D-glucose efflux from preloaded BLV were determined as described in Materials and Methods using a 10-sec efflux period. Vesicles were preloaded with the same concentrations of D-glucose + ^{3}H-D-glucose as used for the uptake experiment in Fig. 4. Carrier-mediated efflux was calculated by correcting for diffusion and the kinetic parameters were determined on each of four batches of BLV as described for kinetics of uptake.

across BLV. Figure 5 shows a Dixon plot of cytochalasin B concentration versus the initial rate of transport-mediated uptake of 1.0 and 2.5 mM D-glucose. The X-value at the point of intersection of the two lines is an estimate of $-K_i$ for the inhibitor. From this experiment, the K_i for cytochalasin B as an inhibitor of D-glucose transport is 0.11 μM.

Characteristics of ^{3}H-Cytochalasin B Binding to BLV

^{3}H-cytochalasin B binding has proven to be a useful method for characterizing and quantifying the D-glucose transporter in numerous cell types such as the red cell and the adipocyte. We found that cytochalasin B is a potent inhibitor of transport across BLV and hence we set out to develop a ^{3}H-cytochalasin B binding assay using BLV.

In order to ensure that ^{3}H-cytochalasin B binding does represent a reliable estimate of D-glucose transporter sites, a number of essential criteria must be established. First, ^{3}H-cytochalasin B is known to interact with multiple classes of binding sites, including the D-glucose transporter. It is possible to selectively inhibit binding to the D-glucose transporter by the addition of D- but not L-glucose. By subtracting ^{3}H-cytochalasin B binding in the presence of D-glucose from total binding in the presence of L-glucose, it is possible to calculate D-glucose-

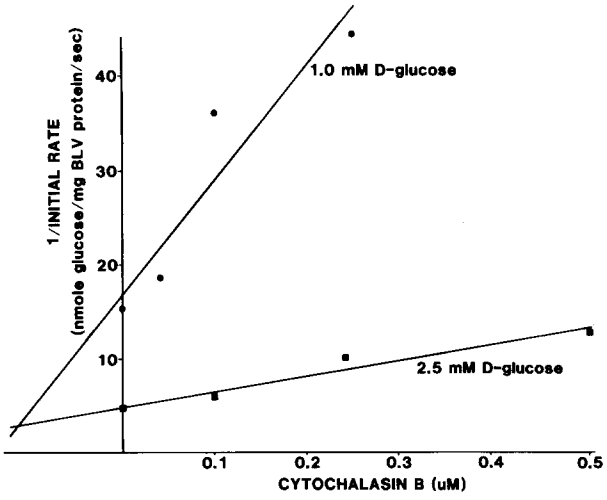

Fig. 5. Dixon plot of the effect of cytochalasin B on the initial rate of 1.0 and 2.5 mM carrier-mediated D-glucose uptake across BLV. Vesicles were preincubated for 5 min at the indicated concentrations of cytochalasin B and initial rates of carrier-mediated uptake were determined as described in Fig. 3. Each point represents the mean value obtained from four determinations of carrier-mediated uptake

inhibitable ^{3}H-cytochalasin B binding. In order to establish that D-glucose-inhibitable ^{3}H-cytochalasin B binding does not underestimate the number of transporters, it is necessary to determine the concentration of D-glucose that causes complete saturation (i.e. maximal inhibition) of the binding sites.

Table 3. Effect of cytochalasin E on the binding of 0.1 μM ^{3}H-cytochalasin to BLV

Cyt E (μM)	Bound cytochalasin B[a] (pmol/mg BLV protein)		L-D
	L-glucose	D-glucose	
0.00	25.97 ± 1.28	21.86 ± 1.21	4.11*
0.50	22.44 ± 1.13	19.42 ± 1.31	3.02
1.00	20.79 ± 1.02	16.36 ± 1.04	4.43*
2.50	18.82 ± 1.05	14.22 ± 1.04	4.60*
5.00	16.20 ± 0.67	13.61 ± 1.01	2.58
10.00	11.65 ± 0.62	12.35 ± 0.55	−0.70

[a] Binding determined in the presence of 1.25 M L- or D-glucose with various concentrations of cytochalasin E added to the incubation media. Values represent the mean ± standard error of a triplicate determination under the indicated conditions.
* Statistically significant L- D-glucose calculation ($P < 0.05$).

Further, it is possible to improve the efficiency of the assay by the addition of cytochalasin E, an analog of cytochalasin B, to the incubation media. Optimal concentrations of cytochalasin E act to decrease ^{3}H-cytochalasin B binding to sites other than the D-glucose transporter.

Table 3 shows the effects of various concentrations of cytochalasin E on the binding of 0.1 μM ^{3}H-cytochalasin B to BLV in the presence of L- and D-glucose. Ten μM cytochalasin E caused a 50% decrease in ^{3}H-cytochalasin B binding; however, this high concentration of cytochalasin E eliminated D-glucose-inhibitable ^{3}H-cytochalasin B binding. Concentrations up to 2.5 μM cytochalasin E were effective in reducing total binding without altering D-glucose-inhibitable binding. We incorporated 2.0 μM cytochalasin E in all subsequent experiments to improve the efficiency of the assay.

Early experiments indicated that D-glucose concentrations up to 500 mM were not sufficient to maximize D-glucose-inhibitable ^{3}H-cytochalasin B binding. Figure 6 shows the effect of very high concentrations of L- and D-glucose on the binding of 0.1 μM ^{3}H-cytochalasin B. At L-glucose concentrations over 500 mM, an increase in binding is seen. This effect is likely related to the osmolarity of the medium and reaches a maximum at 1.0 M L-glucose. Similarly, more ^{3}H-cytochalasin B is bound to the vesicles at high concentrations of D-glucose. However, D-glucose-inhibitable ^{3}H-cytochalasin B binding does increase with glucose concentration and reaches a plateau at concentrations of 1.0 M and greater. Hence, we deemed it necessary to preincubate the vesicles with 1.25 M L- and D-glucose for reliable determinations of the number of D-glucose transporters.

Figure 7 shows a Scatchard plot of D-glucose-inhibitable ^{3}H-cytochalasin B binding data obtained from three separate preparations of BLV. In each experiment, four concentrations of ^{3}H-cytochalasin B were used and ancova analyses of the regression lines for bound versus bound/free indicate no significant differences between preparations. When the data were grouped and a single regression plotted, the number of D-glucose-inhibitable ^{3}H-cytochalasin binding sites was found to be 8.3 ± 1.1 pmol/mg BLV protein and the K_D of ^{3}H-cytochalasin B binding is 0.08 ± 0.02 μM.

Discussion

Susceptibility to proteolytic digestion has been used to determine the orientation of the cytochalasin B-sensitive D-glucose transporter in intact cells, vesicle preparations and after reconstitution in liposomes. Tryptic digestion of intact cells versus inside-out vesicles demonstrates that, in the red cell, the transporter is orientated with a trypsin-sensitive site exposed on the inward-facing surface of the membrane [1]. Shanahan and D'Artel-Ellis [15] have shown that low concentrations of chymotrypsin caused identical fragmentation of the transporter in sealed and unsealed red cell ghosts, suggesting that the transporter has a chymotrypsin cleavage site exposed on the outward-facing surface of the membrane. Further, Chen et al. [3] found that trypsin and chymotrypsin treatment eliminated cytochalasin B binding and transport function in unsealed ghosts. These authors used susceptibility to tryptic and chymotryptic digestion to determine the orientation of the transporter after reconstitution in liposomes. Cytochalasin B binding and D-glucose transport were resistant to trypsin but sensitive to chymotrypsin leading to the conclusion that the transporter retained a right-side-out orientation.

In our experiments with isolated intestinal epithelial cells, we found that phloridzin-insensitive D-glucose transport was sensitive to chymotryptic digestion, while high concentrations of trypsin had little effect on transport. These data suggest that the Na^+-independent D-glucose transporter in the basolateral membrane of the small intestine has a similar structural orientation to that seen in the red cell in that a chymotrypsin-sensitive site is exposed on the outward-facing surface of the membrane while the trypsin cleavage site is located on the inner surface and thus inaccessible to digestion.

One of the objectives of this project was to determine the kinetic parameters for D-glucose transport in both directions across the basolateral membrane. The difficulties inherent in measuring

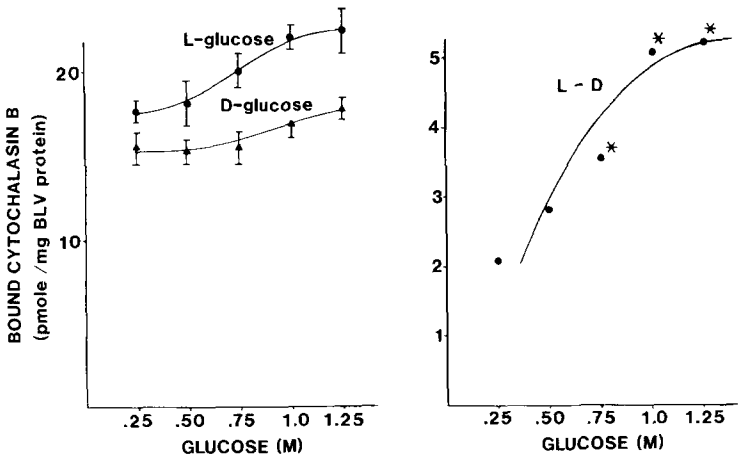

Fig. 6. Effect of varying concentrations of L- and D-glucose on ^{3}H-cytochalasin B binding to BLV. Binding was performed as described in Materials and Methods with 2.0 μM cytochalasin E and 0.1 μM ^{3}H-cytochalasin B incorporated in the mixture. Each value represents the mean ± SE of a triplicate determination and * indicates a significant L-D glucose calculation ($P = 0.05$)

transport across isolated intestinal epithelial cells make it impossible to achieve reliable measures of uptake and efflux kinetics. As such, we used a preparation of purified basolateral membrane vesicles to estimate initial rates of glucose flux across the membrane. If one wishes to extrapolate uptake and efflux kinetics obtained using basolateral vesicles to the situation as it exists in vivo, it is essential to know the orientation of the vesicle preparation. We determined the susceptibility of glucose uptake across our basolateral vesicles to predigestion with trypsin and chymotrypsin. In the four batches of vesicles tested, chymotrypsin inhibited between 75 and 95% of the transport while tryptic digestion decreased transport by 5 to 20%. These data are comparable to that obtained with isolated cells and indicate that the protocol used in this laboratory generates a preparation of predominant right-side-out vesicles.

The kinetics of Na-independent D-glucose transport have been studied extensively in the red blood cell and the adipocyte. The transporter in the red cell is functionally asymmetric, with a K_t for uptake in the range of 1 to 6 mM and a K_t for efflux of four- to 15-fold higher [8, 13]. In the adipocyte, the transporter exhibits much less asymmetry, with a K_t for uptake similar to that seen in the red cell but a K_t for efflux variously reported to be from equal up to twofold higher than that of uptake [6, 16]. The kinetics of D-glucose transport in the basolateral membrane of the small intestine differ from that seen in other cell types. Kimmich and Randles [9] estimated the K_t for phloridzin-insensitive D-glucose uptake in isolated small intestinal epithelial cells to be in the order of 50 to 100 mM. Similarly, Wright et al. [19] reported a K_t of 44 mM for Na-independent transport across purified basolateral membrane vesicles. In our preparation of predominantly right-side-out vesicles, we found an average

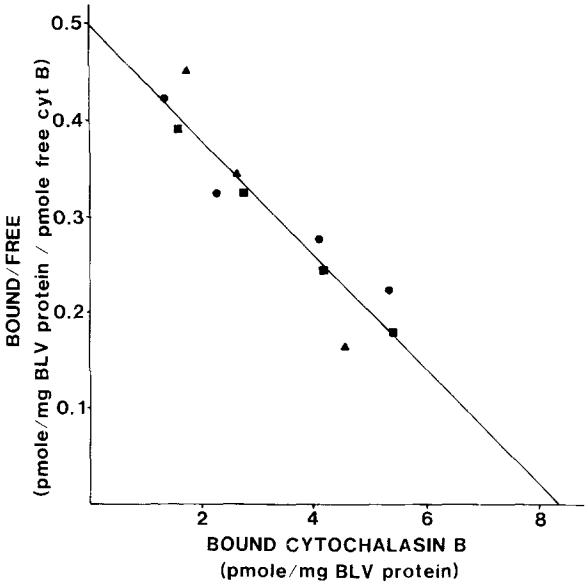

Fig. 7. Scatchard plot of D-glucose-inhibitable ^{3}H-cytochalasin B binding to BLV. For a given preparation of vesicles, triplicate determinations of D-glucose-inhibitable ^{3}H-cytochalasin B binding were performed at each of four concentrations of ^{3}H-cytochalasin B as described in Materials and Methods. All assays were performed using 1.25 M D- or L-glucose and 2.0 μM cytochalasin E. The experiment was repeated on three batches of vesicles and common symbols represent mean values obtained from the same experiment. Ancova analysis indicated no significant difference between regression lines drawn for each experiment. As such, a single line was calculated ($r = -0.94$) which showed saturation of binding sites at 8.3 ± 1.1 pmol/mg BLV protein with a K_D of 0.08 ± 0.02 μM ^{3}H-cytochalasin B

K_t for uptake of 48 mM. These values are approximately 10-fold higher than the K_t for uptake as reported in the red cell. The K_t for efflux from our vesicle preparation averaged 22 mM and is similar to that reported in the red cell.

Detailed studies of ^{3}H-cytochalasin B binding and inhibition of glucose transport by cytochalasin B has led to the specific characterization of the Na-independent D-glucose transporter in the red cell [14, 15], adipocyte [5, 17] and cultured muscle cell [10]. In these cell types, the K_D for D-glucose-inhibitable ^{3}H-cytochalasin B binding approximates 0.1 μM and is similar to the K_i for cytochalasin B inhibition of transport. Such studies indicate that D-glucose-inhibitable ^{3}H-cytochalasin B binding can be used as a specific assay for quantifying the number of transporters in the membrane. In our experiments using intestinal basolateral membrane vesicles, we found a similar K_D for ^{3}H-cytochalasin B binding and K_i for inhibition of D-glucose transport as reported in other cell types. Hence, there appears to be considerable similarity in the cytochalasin B binding site on the D-glucose transporter found in the intestinal basolateral membrane and the red cell and adipocyte plasma membranes. We found the concentration of transporter per mg protein in the intestinal basolateral membrane to be similar to that reported in the adipocyte [5].

The difference in the kinetics of D-glucose transport in basolateral vesicles when compared to the red cell or the adipocyte is reflected in the concentration of D-glucose required to achieve maximum inhibition of ^{3}H-cytochalasin B binding. In our system, we required over 1.0 M D-glucose to maximize D-glucose-inhibitable ^{3}H-cytochalasin B binding, while in the adipocyte, concentrations, between 250 and 500 mM are sufficient for this purpose [5, 17]. This difference correlates with the higher K_t for D-glucose uptake (hence higher K_D for glucose binding) seen in the basolateral membrane.

The kinetics of D-glucose transport in the basolateral membrane of the intestinal epithelium may reflect the specialized function of the tissue. A K_t of 22 mM for efflux from the cell coupled with the action of the Na-dependent transporter in the brush border of the cell may facilitate an efficient system for net transport from lumen to blood. A relatively high K_t for backflux may act to minimize flow from blood to cell cytoplasm and thus improve the efficient of glucose absorption.

This work was supported by the Canadian MRC and the A.M.F.M.R. We would like to thank Ms. D. Devlin for technical assistance and Dr. Ian Simpson for helpful discussions.

References

1. Baldwin, J.M., Lienhard, G.E., Baldwin, S.A. 1980. The monosaccharide transport system of the human erythrocyte. *Biochim. Biophys. Acta* **599**:699–714
2. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* **72**:248–251
3. Chen, C.C., Kurokowa, T., Shaw, S.Y., Jillotson, L.K., Kalled, S., Isselbacher, K.J. 1986. Human erythrocyte glucose transporter: Normal asymmetric orientation and function in liposomes. *Proc. Natl. Acad. Sci. USA* **83**:2652–2656
4. Csaky, T.Z., Fischer, E. 1981. Intestinal sugar transport in experimental diabetes. *Diabetes* **30**:568–574
5. Cushman, S.W., Wardzala, L.J. 1980. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. *J. Biol. Chem.* **255**:4758–4762
6. Gliemann, J., Rees, W.O. 1983. The insulin-sensitive hexose transport system in adipocytes. *Curr. Top. Membr. Transp.* **18**:339–379
7. Jung, C., Rampal, A. 1977. Cytochalasin B binding-sites and glucose-transport carrier in human erythrocyte ghosts. *J. Biol. Chem.* **252**:5456–5463
8. Karlish, S.J.D., Lieb, W.R., Ram, D., Stein, W.O. 1972. Kinetic parameters of glucose efflux from human red blood cells under zero-trans conditions. *Biochim. Biophys. Acta* **255**:126–132
9. Kimmich, G.A., Randles, J. 1979. Energetics of sugar transport by isolated intestinal epithelial cells: Effects of cytochalasin B. *Am. J. Physiol.* **237**:C56–C63
10. Klip, A., Logan, W.J., Li, G. 1982. Hexose transport in L6 muscle cells. Kinetic properties and the number of [^{3}H]cytochalasin B binding sites. *Biochim. Biochim. Acta* **687**:265–280
11. Lin, S., Spudich, J.A. 1974. Binding of cytochalasin B to a red cell membrane protein. *Biochim. Biophys. Res. Commun.* **61**:1471–1476
12. Maenz, D.D., Cheeseman, C.I. 1986. Effect of hyperglycemia on D-glucose transport across the brush border and basolateral membrane of rat small intestine. *Biochim. Biophys. Acta* **860**:277–285
13. Miller, D.M. 1971. The kinetics of selective biological transport. V. Further data on the erythrocyte monosaccharide transport system. *Biophys. J.* **11**:915–923
14. Shanahan, M.F. 1982. Cytochalasin B: A natural photoaffinity ligand for labeling the human erythrocyte glucose transporter. *J. Biol. Chem.* **257**:7290–7293
15. Shanahan, M.F., D'Artel-Ellis, J. 1984. Orientation of the glucose transporter in the human erythrocyte membrane. *J. Biol. Chem.* **259**:13878–13884
16. Taylor, L.P., Holman, G.D. 1981. Symmetrical kinetic parameters for 3-0-methyl-D-glucose transport in adipocytes in the presence and absence of insulin. *Biochim. Biophys. Acta* **642**:325–335
17. Wardzala, L.J., Cushman, S.W., Sulans, L.B. 1978. Mechanism of insulin action on glucose-transport in isolated rat adipose cells-Enhancement of number of functional transport systems. *J. Biol. Chem.* **253**:8002–8005
18. Watford, M., Lund, P., Krebs, H.A. 1979. Isolation and metabolic characteristics of rat and chicken enterocytes. *Biochem. J.* **178**:589–596
19. Wright, E.M., Van Os, C.H., Mircheff, A.K. 1980. Sugar uptake by intestinal basolateral membrane vesicles. *Biochim. Biophys. Acta* **597**:112–124

Received 3 September 1986; revised 27 January 1987