Journal of Biochemistry and Molecular Biology, Vol. 32, No. 5, September 1999, pp. 429–435

JOURNAL OF
Biochemistry
and
Molecular Biology

© BSRK & Springer-Verlag 1999

Investigation of the Nature of the Endogenous Glucose Transporter(s) in Insect Cells

Chong-Kee Lee*
Department of Microbiology, School of Medicine, Catholic University of Taegu-Hyosung, Taegu 705-718, Korea

Received 23 February 1999, Accepted 13 April 1999

Unlike the mammalian glucose transporter GLUT1, little is known about the nature of the endogenous sugar transporter(s) in insect cells. In order to establish the transport characteristics and other properties of the sugar transport proteins of Sf9 cells, a series of kinetic analyses was performed. A saturable transport system for hexose uptake has been revealed in the insect cells. The apparent affinity of this transport system(s) for 2-deoxy-D-glucose was relatively high, the K_m for uptake being <0.5 mM. To further investigate the substrate and inhibitor recognition properties of the insect cell transporter, the ability of other sugars or drugs to inhibit 2-deoxy-D-glucose transport was examined by measuring inhibition constants (K_i). Transport was inhibited by D-mannose, D-glucose, and D-fructose. However, the apparent affinity of the C-4 epimer, D-galactose, for the *Spodoptera* transporter was relatively low, implying that the hydroxyl group at the C-4 position may play a role in the strong binding of glucose and mannose to the transporter. The results also showed that transport was stereoselective, being inhibited by D-glucose but not by L-glucose. It is therefore concluded that insect cells contain an endogenous glucose transport activity that in several aspects resembles the human erythrocyte glucose transporter. However, the mammalian and insect transporters were different in some of their kinetic properties, namely, their affinities for fructose and for cytochalasin B.

Keywords: Glucose transporter, Hexose uptake, Kinetic analysis, Sf9 cell.

* To whom correspondence should be addressed.
Tel: 82-53-650-4477; Fax: 82-53-650-4477
E-mail: leeck@cuth.cataegu.ac.kr

Introduction

The uptake of glucose into mammalian cells is mediated by passive transport proteins in the plasma membrane (Baldwin, 1993; Yoon *et al.,* 1997). Recent evidence has demonstrated that these proteins are members of a large family of homologous sugar transporters found in all organisms, from *Escherichia coli* to plants and animals (Baldwin and Henderson, 1989; Baldwin, 1993). The best known of these proteins is the human erythrocyte glucose transporter, which has been extensively characterized (Baldwin *et al.,* 1982; Mueckler *et al.,* 1985). Functional expression of small amounts of the transporter has been achieved in *Xenopus* oocytes (Gould and Lienhard, 1989) and in mammalian cells (Oka *et al.,* 1990). However, for detailed studies of structure-function relationships in this protein, and in particular for the investigation of its structure by x-ray crystallography, higher levels of expression would be desirable. Recently, the baculovirus/*Spodoptera frugiperda* Clone 9 (Sf9) cell system has been utilized to produce large amounts of the erythrocyte glucose transporter for structural and functional studies (Yi *et al.,* 1992). Characterization of the expressed protein was expected to include assay of its function, including its ability to transport sugars and to bind inhibitory ligands such as cytochalasin B. However, it was not possible to determine the transport activity of the expressed protein in insect cells, because of the presence of endogenous transport systems (Yi *et al.,* 1992). It is therefore interesting to establish the transport characteristics and other properties of the endogenous sugar transport proteins of host insect cells. Very little is known of the transport characteristics of Sf9 cells, although their ability to grow on TC 100 medium strongly suggested the presence of endogenous glucose transporters: this medium contains 0.1% D-glucose as the major carbon source. In the present work, the kinetic properties and sensitivity to inhibitors of the insect cell transporter(s) are characterized.

Materials and Methods

Insect cell culture Sf9 cells were maintained according to the method described by Summers and Smith (1987), with some modification. The cells were cultured in complete TC100 medium [TC100 medium (Gibco-BRL), 10% (v/v) fetal calf serum (Flow), 1% antibiotics (penicillin 5000 units/ml + streptomycin 5000 μg/ml, Gibco-BRL)] at 27°C. Cell viability was checked by adding 0.1 ml of trypan blue (0.4% stock, pH 7.3) to 1 ml of cells and examining under a microscope (Lee, 1979).

Hexose transport assay Tritiated sugars used were 2-(1,2-^{3}H)-deoxy-D-glucose (30.2 Ci/mmol) and L-1-^{3}H(N)]-glucose (10.7 Ci/mmol) and were supplied by NEN (Boston, USA). Prior to 2-deoxy-D-glucose (2dGlc) uptake measurements, Sf9 cells were washed twice with phosphate-buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, pH 7.4) to remove glucose. The cells were resuspended at a density of 1×10^6 cells per ml in PBS and then stored on ice until required for the experiment (up to 30 min). Assays were performed in triplicate, with 1×10^6 cells per assay. The hexose transport assay was performed as follows: 1 ml of the cell suspension was centrifuged at $6000 \times g$ for 15 s in a microfuge and resuspended to a volume of 150 μl in PBS. The cells were then incubated at 27°C for 2 min. Transport was initiated by the addition of 100 μl of 2.5 mM 2dGlc or L-glucose containing 1 μCi tritiated sugar to give a final concentration of 1 mM sugar. Following incubation for 1 min at 27°C, the assay was terminated by adding 1 ml of ice-cold PBS containing 10 μM cytochalasin B and 0.1 mM phloretin, potent glucose transport inhibitors, and then by centrifuging at $12{,}000 \times g$ for 20 s in a microfuge. The cells were washed in this fashion a further two times and then solubilized in 200 μl of 10% SDS by vortexing. Finally, 150 μl of the solubilized cells were dispensed to a vial containing 4 ml of scintillant using a Microman pipette and radioactivity was then determined by liquid scintillation counting (Beckman LS 5,000 CE). All results were expressed as the means of triplicate estimations, which routinely differed from the mean by less than 10%.

Preparation of plasma membranes from Sf9 cells Sf9 cells were cultured as described above. Cells were harvested and washed three times at 20°C with 10 mM-sodium phosphate/150 mM-NaCl, pH7.2. They were then resuspended in 10 mM-Tris/5 mM-$MgCl_2$, pH7.4, containing proteinase inhibitors [2 mM-iodoacetamide, 0.2 mM phenylmethanesulphonyl fluoride and pepstatin A (10 μg/ml)] and sonicated on ice for 1 min. Membranes were separated from soluble components by centrifugation for 1 h at $117{,}000 \times g_{av}$.

Cytochalasin B binding assay The binding of cytochalasin B was used as a functional assay for the endogenous glucose transporter(s) in insect cells and was measured by equilibrium dialysis using [4-^{3}H] cytochalasin B, essentially according to the methods described by Baldwin *et al.* (1982).

Results

Time course of hexose uptake The hexose transport characteristics of Sf9 cells were investigated using 2-[1,2-^{3}H]-deoxy-D-glucose (2dGlc, NEN). To determine the time period over which uptake of sugar into the cells was linear, the uptake of [^{3}H]2dGlc from both low (100 μM) and high (10 mM) extracellular concentrations was measured over periods ranging from 30 s to 30 min. As shown in Figs. 1 and 2, the uptake was linear for at least 2 min at both concentrations, suggesting that measurements made over a 1 min time course would reflect initial rates of the hexose uptake. Additional support for the premise that 1 min uptake experiments provided a measure of the initial rate of uptake was provided by comparison of the intracellular concentration of sugar (calculated from the uptake of radioactivity and the estimated intracellular volume of Sf9 cells) with that outside the cells. For example, when the uptake of 0.1 mM sugar was investigated, the calculated intracellular concentration of sugar corresponded to less than 15% of the extracellular concentration as much as 12 min after uptake had been initiated. Thus, uptake periods of 1 min were used for all further experiments.

Kinetic parameters of the insect cell transporter To determine the K_m (the half-saturation concentration) and V_{max} (the maximum velocity) of the endogenous glucose transporter(s) in the insect cells, the uptake of 2dGlc was measured as described in Materials and Methods, over a range of substrate concentrations (50 μM–10 mM). As can be seen from the data presented in Figs. 3 and 4, 2dGlc uptake by the insect cells appeared to involve both saturable and non-saturable (or very low affinity) components. In contrast, uptake of [1-^{3}H]L-glucose, which

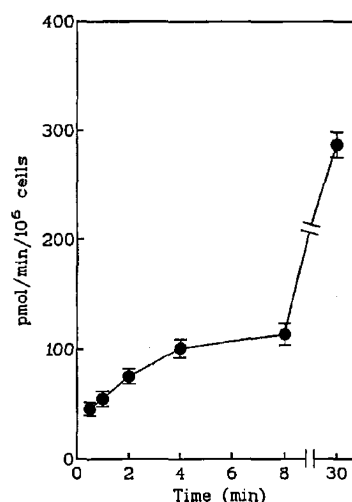

Fig. 1. Time course of 2-deoxy-D-glucose uptake by Sf9 cells from a low extracellular concentration of sugar. Transport was carried out in the presence of 100 μM 2dGlc, as described in Materials and Methods. After the times indicated, uptake was terminated and the radioactivity accumulated was determined by scintillation counting. Each data point on the graph represents the mean of triplicate estimations. The data was corrected for the zero time uptake and converted into pmol/min/10^6 cells.

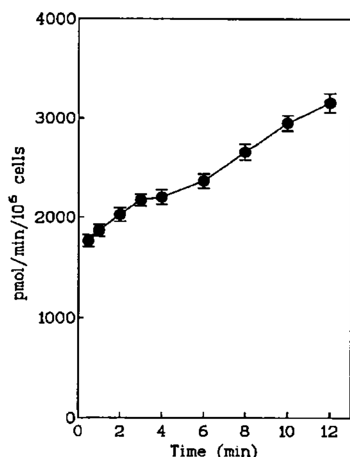

Fig. 2. Time course of 2-deoxy-D-glucose uptake by Sf9 cells from a high extracellular concentration of sugar. Transport was carried out in the presence of 10 mM 2dGlc, as described in the legend to Fig.1. Each data point on the graph represents the mean of triplicate estimations. The data was corrected for the zero time uptake and converted into pmol/min/10^6 cells.

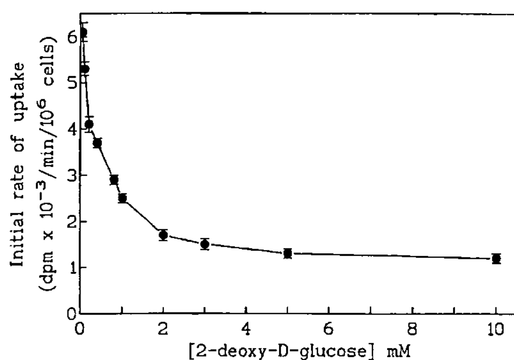

Fig. 3. Transport of 2-deoxy-D-glucose in Sf9 cells. Uptake was carried out as described in Materials and Methods. Shown is a representative experiment in which increasing concentrations of 2dGlc were added to the extracellular medium of the cells. The concentration of radiolabeled 2dGlc used was held constant. Each data point is the mean of triplicate estimations. The K_m for 2dGlc transport was determined with the aid of ENZFITTER, as described in the text.

is not a substrate for the hexose transporters of mammalian cells, appeared to involve only a non-saturable process (Fig. 5). However, the chemical differences between 2dGlc and L-glucose, including the greater hydrophilicity of the L-sugar, precluded use of the latter as a means of correcting the 2dGlc transport data for the non-saturable component of uptake. Instead, the K_m and V_{max} for transport were estimated by using the non-linear regression program ENZFITTER (Elsevier Biosoft, Cambridge, UK) to fit the uptake data to a two-component model described by the following equation:

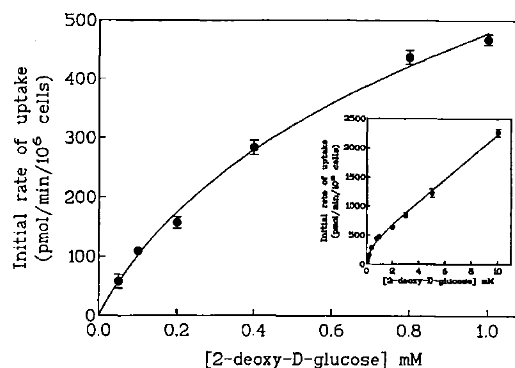

Fig. 4. Determination of the maximal rate of transport (V_{max}). The V_{max} was estimated by using the non-linear regression program ENZFITTER to fit the data to Eq. (1), as described in the text. The curve was produced by using the data points up to 1 mM of 2dGlc, which approach saturation. As shown in the insert, the line drawn through experimental points at high concentrations (> mM) of substrate is straight and represents the non-saturable component.

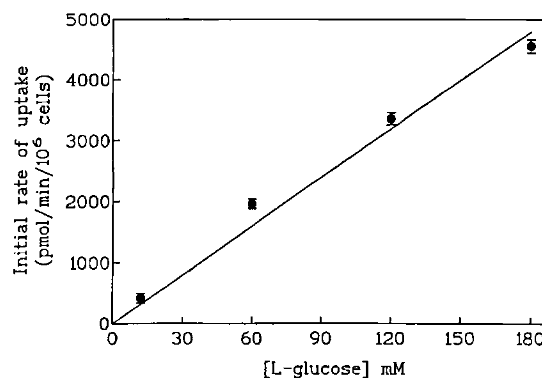

Fig. 5. Uptake of [^{3}H]L-glucose by Sf9 cells. Transport was carried out as described in the Materials and Methods section. Shown is a representative experiment in which increasing concentrations of L-glucose were added to the extracellular medium of the cells. Each data point is the mean of triplicate estimations.

$$V = \frac{V_{max} \bullet S}{K_m + S} + M \bullet S \qquad (1)$$

where V = the rate of uptake, S = substrate concentration, and M = a constant describing the non-saturable component of the uptake process. In the uptake experiments, the concentration of radiolabeled sugar was varied. The resultant plot of radiolabel uptake versus concentration of unlabeled sugar is shown in Fig. 3. The K_m for transport was estimated by using ENZFITTER to fit these data to Eq. (2) (see below). This procedure yielded an apparent K_i for the unlabeled sugar, which corresponds

to the K_m value for 2dGlc uptake, in this case of 378 ± 42 μM. The K_m value was calculated from the raw uptake data (dpm) in this way, rather than by fitting the initial rate data to a hyperbola as described for V_{max} below, in order to avoid undue biasing of the calculation by the uptakes measured at high sugar concentrations. Such biasing would result from the fact that the specific radioactivity of the 2dGlc was not held constant over the range of sugar concentrations used in the present experiment. The V_{max} for transport was then estimated by using ENZFITTER to fit the data from a plot of initial rate of 2dGlc uptake versus substrate concentration (Fig. 4) to Eq. (1), using the K_m value estimated as described above. This procedure yielded a V_{max} value of 415 ± 37 pmol/min/10^6 cells. In addition, the value of M (218) calculated from the computer fit to Eq. (1) for the non-saturable component of the uptake process corresponded closely to that (209) directly estimated from the slope of the curve shown in Fig. 4 at high 2dGlc concentrations. For comparison, the V_{max} of the endogenous transporter in insect cells for 2dGlc was about 9.7-fold higher than that calculated for Chinese hamster ovary (CHO) cells which contain a GLUT1 homologue, whereas the K_m value of the insect transporter for 2dGlc was approximately 1.4-fold lower than that reported for the latter cells (Ishihara *et al.*, 1991). Thus, it appeared that the transport activity of the insect cells was very high.

Studies of the inhibition of 2dGlc transport in insect cells by hexoses, phloretin, and cytochalasin B The kinetic experiments described above had revealed the existence of a saturable transport system for hexose uptake by insect cells. The apparent affinity of this transport(s) for 2-deoxy-D-glucose was relatively high, the K_m for uptake being < 0.5 mM. To investigate further the substrate and inhibitor recognition properties of the insect cell transporter, the ability of other sugars and drugs to inhibit 2-deoxy-D-glucose transport was examined by measuring inhibition constants (K_i). Transport assays were carried out essentially as described in Materials and Methods. To initiate transport, cells were added to 100 μl of PBS buffer containing radiolabeled substrate and an appropriate concentration of inhibitor. After the required time of 1 min, the transport was terminated and radioactivity was determined.

The effect of a competitive inhibitor on the uptake of 2dGlc can be described by the following equation:

$$V_I = \frac{V_{max} \cdot S}{K_m \cdot (1 + I/K_i) + S} + M \cdot S \qquad (2)$$

where V_I = the rate of uptake in the presence of inhibitor,
I = inhibitor concentration,

and the other terms are as described for Eq. (1).

The K_i's for reversible inhibitors were determined from plots of uptake versus inhibitor concentration. For each inhibitor illustrated in Figs. 6–12, the curve through the data points represents the best fit of the data to Eq. (2). Using this approach, 2dGlc uptake in the insect cells was found to be most potently inhibited by phloretin, the aglucone of phlorizin, which inhibited with an apparent K_i of 0.77 ± 0.10 μM (Fig. 6). Next in order of inhibitory potency was cytochalasin B (apparent K_i = 9.8 ± 7.1 μM, Fig. 7). The cytochalasin B was dissolved in ethanol and the concentration of ethanol was kept constant in all the samples. Of the hexoses tested, L-glucose had the least effect on 2dGlc transport in the insect cells (apparent K_i = 50.1 ± 32.7 mM, Fig. 8), indicating that the transport is stereoselective. D-Mannose had the highest apparent affinity for the insect cell transporter, with K_i = 1.24 ± 0.23 mM (Fig. 9). Unlike the situation for the human erythrocyte glucose transport system, where the K_d for mannose is somewhat greater than that for glucose, this apparent affinity of the C-2 epimer for the insect cell transporter was similar to that of D-glucose itself (K_i = 1.46 ± 0.28 mM, Fig. 10). However, as shown in Fig. 11, epimerization at the C-4 position of D-glucose resulted in a dramatic decrease in affinity of the hexose for the insect cell transporter; the apparent K_i for inhibition of 2dGlc transport by D-galactose being 24.5 ± 6.8 mM. Interestingly, the insect cell sugar transporter also showed high affinity for D-fructose, which inhibited 2dGlc transport with an apparent K_i = 4.33 ± 0.90 mM (Fig. 12). In contrast, the reported K_m of the human erythrocyte transporter for the ketose is 1.5 M (LeFevre and Marshall, 1958).

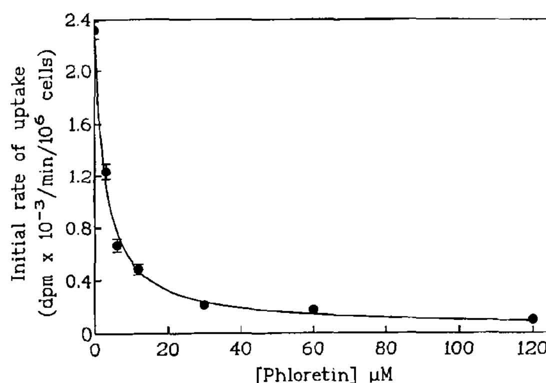

Fig. 6. Effect of phloretin on the initial rate of 2-deoxy-D-glucose uptake. Transport was initiated by the addition of 100 μl of PBS buffer containing [^{3}H]2dGlc and an appropriate concentration of phloretin. After 1 min, the transport was terminated by addition of ice-cold stop solution. Cells were then solubilized and radioactivity was determined as described in Materials and Methods. The data were analyzed with the help of ENZFITTER. The curve represents the best fit of the data to Eq. (2). Each data point represents the mean of triplicates.

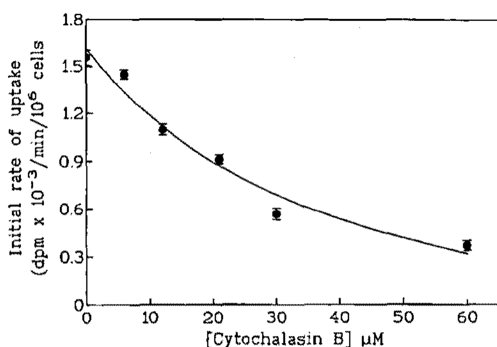

Fig. 7. Effect of cytochalasin B on 2-deoxy-D-glucose transport. Transport assay was carried out essentially as described in Materials and Methods. The ability of cytochalasin B to inhibit the uptake of 2dGlc was determined as described in the legend to Fig. 6. The curve represents the best fit of the data to Eq. (2). Each data point is the mean of triplicate estimations.

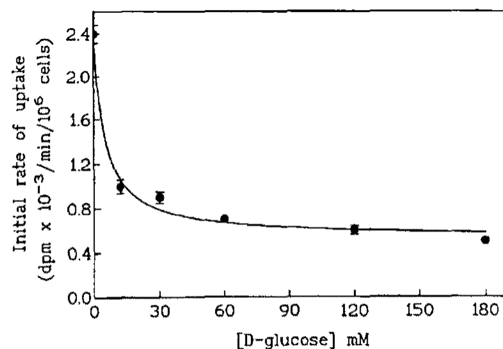

Fig. 10. Effect of D-glucose on 2-deoxy-D-glucose transport. Transport assay was carried out essentially as described in Materials and Methods. The ability of D-glucose to inhibit the uptake of 2dGlc was determined as described in the legend to Fig. 6. The curve represents the best fit of the data to Eq. (2). Each data point is the mean of triplicate estimations.

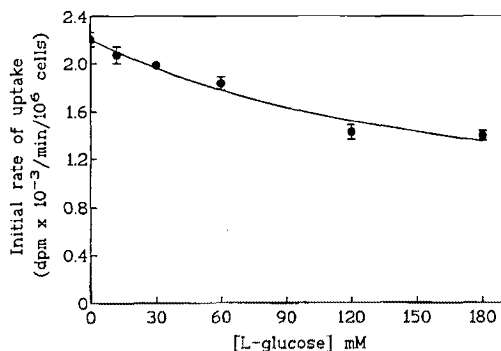

Fig. 8. Effect of L-glucose on 2-deoxy-D-glucose transport. Transport assay was carried out essentially as described in Materials and Methods. The ability of L-glucose to inhibit the uptake of 2dGlc was determined as described in the legend to Fig. 6. The curve represents the best fit of the data to Eq. (2). Each data point is the mean of triplicate estimations.

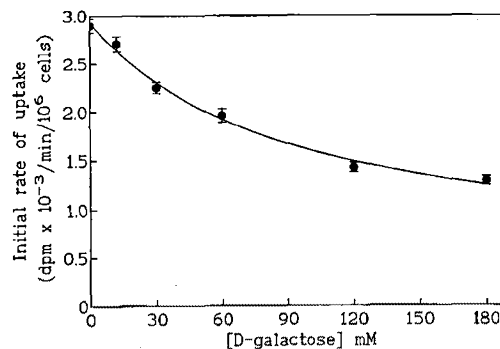

Fig. 11. Effect of D-galactose on the initial rate of 2-deoxy-D-glucose uptake. Transport assay was carried out essentially as described in Materials and Methods. The ability of D-galactose to inhibit the uptake of 2dGlc was determined as described in the legend to Fig. 6. The curve represents the best fit of the data to Eq. (2). Each data point is the mean of triplicate estimations.

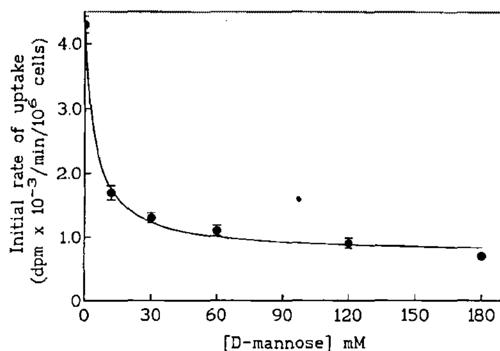

Fig. 9. Effect of D-mannose on 2-deoxy-D-glucose transport. Transport assay was carried out essentially as described in Materials and Methods. The ability of D-mannose to inhibit the uptake of 2dGlc was determined as described in the legend to Fig. 6. The curve represents the best fit of the data to Eq. (2). Each data point is the mean of triplicate estimations.

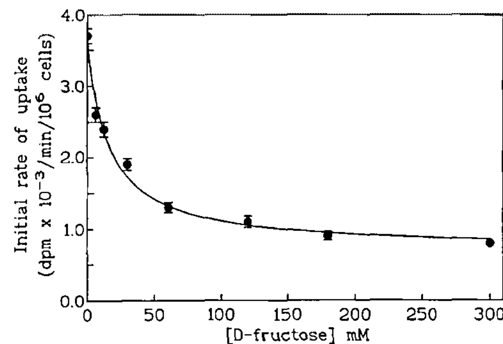

Fig. 12. Effect of D-fructose on 2-deoxy-D-glucose transport. Transport assay was carried out essentially as described in Materials and Methods. The ability of D-fructose to inhibit the uptake of 2dGlc was determined as described in the legend to Fig. 6. The curve represents the best fit of the data to Eq. (2). Each data point is the mean of triplicate estimations.

Cytochalasin B binding to insect cell membranes Cytochalasin B is a potent inhibitor of the human erythrocyte glucose transporter, to which it binds with a K_d of approximately 0.12 μM (Baldwin *et al.*, 1982). The experiments described above showed that it was a much less potent inhibitor of the hexose transport in insect cells. However, it was not clear whether this lower potency resulted from a lower affinity of the transporter for the inhibitor, or from a lesser effect of bound inhibitor on the transport activity of the protein. In order to resolve this question, the ability of cytochalasin B to bind to the insect cell transporter was directly examined in a ligand binding assay. Cytochalasin B binding activity was assayed by equilibrium dialysis using a single, final concentration of [4-^{3}H] cytochalasin B of 40 nM, in the absence and presence of 400 mM D-glucose (Baldwin *et al.*, 1982). The insect cell membranes used were prepared as described in Materials and Methods. Alkali-stripped human erythrocyte membranes (Gorga and Lienhard, 1981) were used as a positive control. Cytochalasin binding activity was calculated by subtracting the value of the ratio of bound cytochalasin B to free cytochalasin B obtained in the presence of D-glucose from the equivalent value obtained in the absence of D-glucose (Yi *et al.*, 1992). The corrected bound-to-free ratio is approximately equal to the ratio of the concentration of cytochalasin B binding sites on glucose transporters to the dissociation constant for cytochalasin B. Thus, it is proportional to the concentration of binding sites (Baldwin *et al.*, 1982). The cytochalasin B binding activity for the insect cell and erythrocyte membranes at the concentration of 1 mg/ml were 0.001 and 7.538, respectively (Table 1). From the result, it may be concluded that the absence of the binding activity in the former results either from the low concentration of binding sites on the insect transporter(s), or from the low affinity for the transporter, or both. However, judging from the effects on transport above, it is probably because of low affinity. Therefore, the presence of endogenous transporters should not interfere with the assay of the cytochalasin B binding properties of human GLUT1 expressed in insect cells.

Table 1. Cytochalasin B binding to Sf9 cell membranes.

Sample (1 mg/ml)	Cytochalasin B (B/F) (−) D-Glucose	(+) D-Glucose	*Specific B/F
Sf9 cell membranes	0.049	0.048	0.001
Erythrocyte membranes**	8.055	0.517	7.538

The binding of cytochalasin B was measured at a single low concentration (40 nM), in the absence (−) and presence (+) of 400 mM D-Glucose, as described previously (Baldwin *et al.*, 1982). *Cytochalasin B binding activity was calculated as described before (Gorga and Lienhard, 1981). **Human erythrocyte membranes were prepared as described previously (Gorga and Lienhard, 1981). B/F = [bound cythochalasin B]/[free cytochalasin B].

Discussion

The erythrocyte glucose transporter has a broad substrate specificity, with a large number of simple sugars being substrates. The rank order of apparent affinities is as follows: 2-deoxy-D-glucose > D-glucose > D-mannose > D-galactose > D-xylose > L-arabinose > D-fucose > L-fucose > L-rhamnose >> L-glucose (LeFevre, 1961). As is apparent from this rank order, the transporter displays a particularly strong specificity for D-stereoisomers of sugars.

However, unlike the mammalian transporter, little is known about the nature of the sugar transporter(s) in insect cells. The affinities of the insect cell transporter for different hexoses had been examined by measuring their ability to inhibit 2-deoxy-D-glucose (2dGlc) transport in Sf9 cells. Since the same substrate (2dGlc) was used for all experiments of this type, then the apparent K_i values for each sugar should provide a measure of their relative affinities. The uptake of 2dGlc in the Sf9 cells followed a simple linear function of time for at least 2 min. The transport was inhibited by D-mannose, D-glucose, and D-fructose. However, the apparent affinity of the C-4 epimer, D-galactose, for the *Spodoptera* transporter was relatively low, implying that the hydroxyl group at the C-4 position may play a role in the strong binding of glucose and mannose to the transporter. The results also showed that transport was stereoselective, being inhibited by D-glucose but not L-glucose. Unlike the human erythrocyte glucose transport system, which has a very low affinity for fructose (LeFevre and Marshall, 1958), the insect cell transporter appeared to have high affinity for D-fructose (K_i = approximately 5 mM). Indeed, the K_i for fructose was only about 3-fold lower than that for D-glucose, suggesting that the insect cell transporter may also be capable of transporting fructose, although this suggestion would have to be confirmed by transport experiments using radiolabeled fructose itself. Whether or not fructose is in fact transported, its affinity for the transporter suggested that it might be possible to use the ketose as an inhibitor for blocking the endogenous glucose transport activity of insect cells when the functional activity of heterologously-expressed human erythrocyte glucose transporter was being assessed. The insect cell transporter was also found to differ from the human protein in being much less sensitive to inhibition by cytochalasin B. However, it was inhibited by the diphenolic compound phloretin with a K_i (approximately 1 μM) similar to the value of about 2 μM reported for inhibition of glucose transport in human erythrocytes (LeFevre and Marshall, 1959).

The importance of molecular conformation in sugar transport across the lipid bilayer of human erythrocytes was first recognized by LeFevre (1961), who demonstrated a correlation between affinity and propensity to exist in the C1 conformation. It is now thought that the affinity of sugars for the transporter is probably determined by their possession of suitable equatorial hydroxyl groups to form hydrogen bonds with the transporter. Binding affinity is also likely to be influenced by axial hydroxyls that may sterically hinder sugar binding to the transporter. Thus, of the natural hexose and pentose sugars, the β-anomers of D-glucose and D-xylose, which are completely free of axial substituents in the C1 conformation, show the highest affinities for the transport protein, whereas their enantiomers (mirror images), L-glucose and L-xylose, have extremely low affinities for the protein. In addition, epimerization of the equatorial hydroxyl groups at carbons 2, 3, or 4 of D-glucose to an axial orientation is known to result in a marked decrease site of the erythrocyte transporter (LeFevre, 1961). For example, D-galactose (the C-4 epimer of D-glucose) inhibits sorbose transport (via the glucose transporter) with a 13-fold lower affinity than does D-glucose. Similarly, the K_i of the C-4 epimer is 16-fold greater than for D-glucose in the insect cell transport system. Such a lowering of affinity might be the result either of the involvement of the C-4 hydroxyl in hydrogen bonding or of steric hindrance from the axial hydroxyl group. Interestingly, unlike the human erythrocyte glucose transport system, the C-2 epimer (D-mannose) has a somewhat greater affinity for the insect cell transporter than D-glucose. In addition, the kinetic results suggest that 2-deoxy-D-glucose binds more tightly than D-glucose. This finding implies that the hydroxyl group at the C-2 position is not required for strong binding.

In conclusion, insect cells were found to contain an endogenous glucose transport activity that in several aspects resembles the mammalian passive glucose transporter, GLUT1. The presence of such an activity might interfere with assessment of the kinetic properties of the mammalian transporter expressed in the insect cells, although the mammalian and insect transporters do differ in some of their kinetic properties, namely their affinities for fructose and for cytochalasin B. Therefore, it would probably be required to purify the expressed protein away from the endogenous transporter and then reconstitute it into lipid vehicles before detailed kinetic studies could be performed.

Acknowledgments The author would like to thank Dr. S. A. Baldwin (University of Leeds, U.K.) for his support and valuable discussions. This work was supported in part by a fund from the Medical Research Institute of Kyungpook National University Hospital.

References

Baldwin, S. A. (1993) Mammalian passive glucose transporters: members of a ubiquitous family of active and passive transport proteins. *Biochim. Biophys. Acta* **1154**, 17–49.

Baldwin, S. A., Baldwin, J. M. and Lienhard, G. E. (1982) Monosaccharide transporter of the human erythrocyte: Characterization of an improved preparation. *Biochemistry* **21**, 3836–3842.

Baldwin, S. A. and Henderson, P. J. F. (1989) Homologies between sugar transporters from eukaryotes and prokaryotes. *Annu. Rev. Physiol.* **51**, 459–471.

Gorga, F. R. and Lienhard, G. E. (1981) Equilibria and kinetics of ligand binding to the human erythrocyte glucose transporter. Evidence for an alternating conformation model for transport. *Biochemistry* **20**, 5108–5113.

Gould, G. W. and Lienhard, G. E. (1989) Expression of a functional glucose transporter in *Xenopus* oocytes. *Biochemistry* **28**, 9447–9452.

Ishihara, H., Asano, T., Katagiri, H., Lin, J. L., Tsukuda, K., Shibasaki, Y., Yazaki, Y. and Oka, Y. (1991) The glucose transport activity of GLUT1 is markedly decreased by substitution of a single amino acid with a different charge at residue 415. *Biochem. Biophys. Res. Commun* **176**, 922–930.

Lee, H. H. (1979) Identification of baculovirus variants by restriction endonucleases *J. Biochem. Mol. Biol.* **12**, 220.

LeFevre, P. G. (1961) Sugar transport in the red blood cell: structure-activity relationships in substrates and antagonists. *Pharmacol. Rev.* **13**, 39–70.

LeFevre, P. G. and Marshall, J. K. (1958) Conformational specificity in a biological sugar transport system. *Am. J. Physiol.* **194**, 333–337.

LeFevre, P. G. and Marshall, J. K. (1959) The attachment of phloletin and analogues to human erythrocyte in connection with inhibition of sugar transport. *J. Biol. Chem.* **234**, 3022–3026.

Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M. and Blench, I. (1985) Sequence and structure of a human glucose transporter. *Science* **229**, 941–945.

Oka, Y., Asano, T., Shibasaki, Y., Lin, J. L., Tsukuda, K., Katagiri, H., Akanuma, Y. and Takaku, F. (1990) C-terminal truncated glucose transporter is locked into an inward-facing form without transport activity. *Nature* **345**, 550–553.

Summers, M. D. and Smith, G. E. (1987) A manual of methods for baculovirus vectors and insect cell culture procedures. *Tex. Agric. Exp. Stn. Bull.* No. 1555.

Yi, C. K., Charalambous, B. M., Emery, V. C. and Baldwin, S. A. (1992) Characterization of functional human erythrocyte-type glucose transporter expressed in insect cells using a recombinant baculovirus. *Biochem. J.* **283**, 643–646.

Yoon, H. J., Lee, M. Y. and Jhon, G. J. (1997) Effect of ganglioside GM3 on the erythrocyte glucose transporter (GLUT1): Conformational changes measured by steady-state and time-resolved fluorescence spectroscopy. *J. Biochem. Mol. Biol.* **30**, 240–246.