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Redefining the Facilitated Transport of Mannose in Human Cells: Absence of a Glucose-Insensitive, High-Affinity Facilitated Mannose Transport System[†]

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ABSTRACT: Current evidence suggests that extracellular mannose can be transported intracellularly and utilized for glycoprotein synthesis; however, the identity and the functional characteristics of the transporters of mannose are controversial. Although the glucose transporters are capable of transporting mannose, it has been postulated that the entry of mannose in mammalian cells is mediated by a transporter that is insensitive to glucose [Panneerselvam, K., and Freeze, H. (1996) *J. Biol. Chem.* 271, 9417–9421] or by a transporter induced by cell treatment with metformin [Shang, J., and Lehrman, M. A. (2004) *J. Biol. Chem.* 279, 9703–9712]. We performed a detailed analysis of the uptake of mannose in normal human erythrocytes and in leukemia cell line HL-60. Short uptake assays allowed the identification of a single functional activity involved in mannose uptake in both cell types, with a K_m for transport of 6 mM. Transport was inhibited in a competitive manner by classical glucose transporter substrates. Similarly, the glucose transporter inhibitors cytochalasin B, genistein, and myricetin inhibited mannose transport by 100%. Using long uptake experiments, we identified a second, high-affinity component associated with the intracellular trapping of mannose in the HL-60 cells that is not directly involved in the transport of mannose via the glucose transporters. Thus, the transport of mannose via glucose transporters is a process which is kinetically and biologically separable from its intracellular trapping. A general survey of human cells revealed that mannose uptake was entirely blocked by concentrations of cytochalasin B that obliterates the activity of the glucose transporters. The transport and inhibition data demonstrate that extracellular mannose, whose physiological concentration is in the micromolar range, enters cells in the presence of physiological concentrations of glucose. Overall, our data indicate that transport through the glucose transporter is the main mechanism by which human cells acquire mannose.

Analysis of the uptake of mannose in human cells has revealed the presence of two functional activities with different affinities for mannose (1–3), an observation that was interpreted as indicating the existence of at least two separate transport systems involved in the cellular uptake of mannose (3–5). The facilitative glucose transporters, a family of membrane proteins that transport glucose down a concentration gradient, are capable of transporting mannose (6). Given the presence of glucose transporters on all cells and tissues, it is tempting to postulate that these proteins are the universal transporters of mannose in mammalian cells. The glucose

transporters transport mannose with a K_m for transport in the millimolar range. However, data indicating that mammalian cells possess a high-affinity mannose transporter with a K_m in the micromolar range, which is similar to the concentration of mannose in serum, have been published (3). Likewise, the existence of a high-affinity metformin-stimulated D-mannose transport activity has been recently proposed (7). The activity of the high-affinity mannose transporter is insensitive to the concentrations of glucose normally presented in blood. Thus, it has been postulated that the high-affinity transporter, and not the glucose transporters, is the physiologically relevant mannose transporter in mammalian cells.

The data that led to the identification of a high-affinity mannose transporter were obtained from studies in which uptake was assessed in cells incubated with mannose for long uptake periods (3–5, 7, 8). Because no attempt was made to establish the identity of the rate-limiting step for uptake under the conditions of the experiment, it is not valid to assign the kinetic properties of the overall mannose uptake process directly to the transport step. The rate of the cellular

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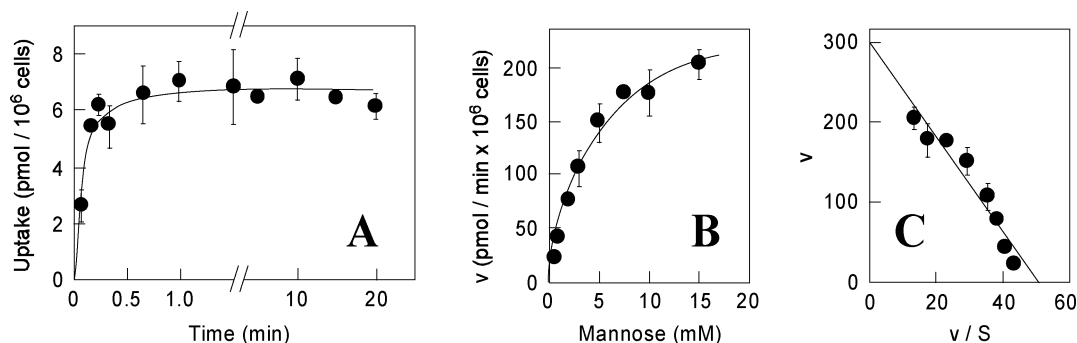


FIGURE 1: Kinetics of mannose uptake by normal human erythrocytes. (A) Time course of uptake of 1 mM mannose. (B) Substrate dose dependence of mannose transport using 5 s uptake assays. (C) v vs v/S plot of the substrate dose dependence of mannose transport from the data in panel B. The line corresponds to a linear regression fit carried out with Sigmaplot 8.0.

uptake of mannose in long uptake experiments is a complex function of both transport and intracellular trapping. Only under initial velocity conditions (using short uptake experiments) can the rate of transport be estimated to be different from the rate of trapping (9). Thus, a better understanding of the mechanisms that regulate the cellular content of mannose requires the identification of the events involved in its transport as well as in trapping. We analyzed the uptake of mannose in normal human erythrocytes and in leukemia cell line HL-60 under experimental conditions that allowed us to distinguish the transport of mannose from its intracellular trapping. We provide evidence indicating that only one transport system, with the functional properties expected for the glucose transporter GLUT1 present in these cells, is directly involved in the facilitated transport of mannose in human erythrocytes and the HL-60 cells. A second functional activity identified in long uptake experiments is associated with the intracellular trapping of mannose and does not represent transport via the glucose transporters. We also provide data indicating that mannose is transported by glucose transporters in a variety of human cells.

EXPERIMENTAL PROCEDURES

Human erythrocytes were purified from outdated blood samples donated by the Blood Bank Service of the Regional Hospital in Valdivia, Chile. HL-60 cells were cultured in Iscove modified Dulbecco's medium supplemented with 10% fetal bovine serum and antibiotics. Cell viability was greater than 95%, as determined by trypan blue exclusion.

For uptake assays, the cells were suspended in incubation buffer [15 mM Hepes (pH 7.6), 135 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, and 0.8 mM MgCl₂], washed by centrifugation, and resuspended at densities of $1\text{--}5 \times 10^7$ cells/mL ($2\text{--}3 \times 10^8$ red cells/mL) (9, 10). Normal human mammary cells, fibroblasts, and umbilical vein endothelial cells were obtained from Clonetics (San Diego, CA), and cultured using their media and supplements. MCF-7 human breast cancer cells and MDCK dog renal cells were from ATCC and were cultured in DMEM supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, and penicillin/streptomycin. Fresh normal human neutrophils were isolated using Ficoll-Hypaque (11). For adherent cells, mannose uptake experiments were performed in six-well plates with cells at 95% confluency. Uptake assays were performed at room temperature in 0.2 mL of incubation buffer containing $1\text{--}10 \times 10^6$ cells ($40\text{--}60 \times 10^6$ cells when using erythrocytes), 1 μ Ci of [³H]mannose (5 Ci/mmol, NEN-

DuPont, Wilmington, DE), and a final concentration of 0.005–15 mM mannose. Uptake was stopped with 5 volumes of cold phosphate-buffered saline containing 0.1 mM HgCl₂. The cells were collected and washed twice by centrifugation in cold stopping solution, solubilized in 10 mM Tris-HCl (pH 8.0) containing 0.2% SDS, and processed for scintillation counting. Hexose uptake assays were performed using 1 μ Ci of 3-*O*-[methyl-³H]-D-glucose (10 Ci/mmol, NEN-Dupont) and 0.3–20 mM 3-*O*-methyl-D-glucose (methylglucose) or 1 μ Ci of 2-[1,2-³H(N)]deoxy-D-glucose (26.2 Ci/mmol, NEN-Dupont) and 0.3–20 mM 2-deoxy-D-glucose (deoxyglucose). Competitors and inhibitors were added to the uptake assays without a preincubation period from concentrated stock solutions. In all cases, data represent the mean \pm standard deviation of four experiments.

Unsealed erythrocyte ghosts were prepared from washed red cells (12). D-Glucose inhibitable binding of cytochalasin B to functional glucose carriers was calculated from the difference between the amount of cytochalasin B bound in the presence of 500 mM L-glucose and 500 mM D-glucose. Binding assays were performed in a final volume of 150 μ L containing 0.06–0.1 mg/mL erythrocyte membrane protein (from $1\text{--}1.6 \times 10^8$ cells), 10 μ M cytochalasin E, 500 mM D- or L-glucose, 0.02–0.04 μ Ci of [4-³H]cytochalasin B (11.9 Ci/mmol, NEN-DuPont), and 0.01–20 μ M cold cytochalasin B. After 10 min at room temperature, the membranes were collected by centrifugation at 15000g for 10 min. The amount of bound cytochalasin B was calculated from the quantity of radioactive ligand associated with the membrane pellet and from the difference in the amount of soluble radioactivity before and after centrifugation (13). Data represent the mean \pm standard deviation of three independent determinations.

RESULTS

Uptake of Mannose by Human Erythrocytes. In initial studies, we developed experimental conditions that would allow us to distinguish the transport of mannose from the process of intracellular trapping of the transported substrate. Differentiating between transport and trapping is accomplished experimentally by carrying out very short uptake assays as opposed to long-term assays (9, 14). When the time course of the mannose uptake was measured in normal erythrocytes, the amount of cell-associated radioactivity increased rapidly at short incubation times and the uptake rate was linear for the first 10 s of incubation (Figure 1A). Thereafter, the rate of incorporation decreased rapidly and reached a plateau that was maintained from 15 s for the

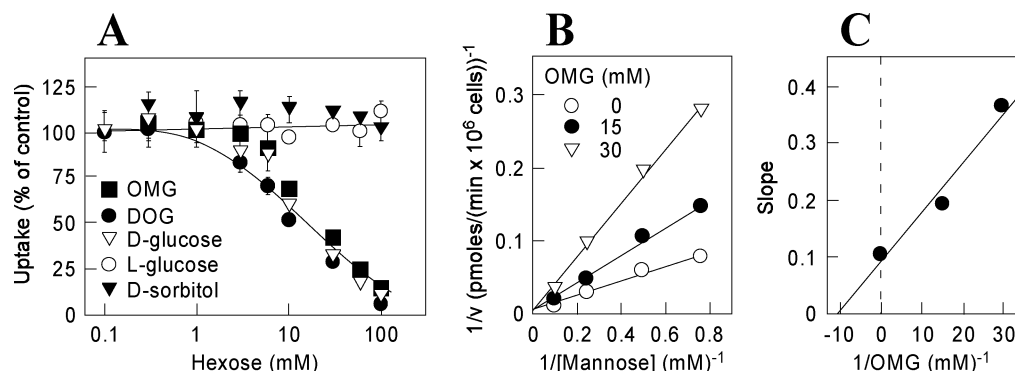


FIGURE 2: Effect of glucose transporter substrates on the transport of mannose by normal human erythrocytes. (A) Concentration dependence for the inhibition of mannose transport by D-glucose (▽), 2-deoxy-D-glucose (●), 3-O-methyl-D-glucose (■), L-glucose (○), or sorbitol (▼). Measurements were performed at 1 mM mannose using 5 s uptake assays. (B) Double-reciprocal plot of the substrate dependence for mannose transport in the absence (○) or in the presence of 15 (●) or 30 mM (▽) 3-O-methyl-D-glucose. (C) Secondary plot of the effect of 3-O-methyl-D-glucose on the substrate dependence for the transport of mannose. Abbreviations: DOG, 2-deoxy-D-glucose; OMG, 3-O-methyl-D-glucose.

length of the incubation period (20 min). We interpreted these results as indicating that the initial phase of incorporation of mannose by the erythrocytes represented the transport of mannose that then equilibrated rapidly without evidence of intracellular trapping. Dose-response studies using 5 s uptake assays revealed that mannose transport by the human erythrocyte cells approached saturation at ~15 mM (Figure 1B). These studies revealed the presence of a single component with an apparent K_m of 6 mM, and a V_{max} of 0.3 nmol/min per 10^6 cells for the transport of mannose by the erythrocytes (Figure 1C).

The concept for the existence of a mannose transporter distinct from the glucose transporters originated in evidence indicating that the uptake of mannose in several cell types was insensitive to the presence of glucose; 0.5 mM glucose had no effect on the uptake of mannose (3, 7). However, because the K_m for the transport of glucose by the most widely expressed glucose transporter, GLUT1, is ~5 mM, it is not expected that 0.5 mM glucose will have a major effect on the transport of a substrate that enters cells through GLUT1. Only concentrations of glucose of ≥ 5 mM should have a clear effect on mannose uptake if it is correct that mannose is transported by a glucose transporter. We addressed this issue by testing the effect of methylglucose, deoxyglucose, and D-glucose on the uptake of mannose by the erythrocytes and found that at 0.5 mM they had a very small effect on the uptake of mannose (Figure 2A). As expected, the effect of the hexoses on mannose transport was dose-dependent; at 100 mM, they blocked mannose uptake by nearly 100%, and 50% inhibition of uptake was observed at hexose concentrations of 8–12 mM (Figure 2A). The specificity of the inhibitory effect is demonstrated by the lack of action of L-glucose and D-sorbitol, two hexoses that are unable to interact with the glucose transporters (Figure 2A). We further analyzed this issue by characterizing in full detail the dose dependency of the effect of methylglucose on the transport of mannose by the human erythrocytes. The competition experiments revealed that methylglucose inhibited mannose transport in a competitive manner (Figure 2B), with a K_i of 10 mM (Figure 2C).

Glucose transporter inhibitors were very effective in inhibiting mannose uptake in human erythrocytes (Figure 3A). The isoflavone genistein and the flavone myricetin, which inhibit GLUT1 function by directly interacting with

the transporter, blocked mannose uptake in human erythrocytes in a dose-dependent manner (Figure 3A). Maximal inhibition of mannose transport was observed at concentrations of $\geq 30 \mu\text{M}$, which is consistent with the known inhibitory effects of these compounds on GLUT1 (10, 13). Cytochalasin B, but not cytochalasin E, inhibited mannose transport in a dose-dependent manner, with more than 100% inhibition observed at 10 μM cytochalasin B (Figure 3A), which is consistent with the affinity of cytochalasin B for GLUT1 (15, 16). In further experiments, we studied the effect of mannose on binding of cytochalasin B to GLUT1 present in erythrocyte membranes. Increasing concentrations of mannose efficiently competed for the glucose-displaceable cytochalasin B binding sites present in the erythrocyte membranes (Figure 3B). Approximately 100 mM mannose inhibited cytochalasin B binding by 50%, while >90% inhibition was observed at 400 mM mannose (Figure 3B). Control experiments revealed that D-glucose inhibited cytochalasin B binding with an IC_{50} of 60 mM, while L-glucose had no effect (Figure 3B). Further analysis revealed that mannose displaced cytochalasin B in a competitive manner (Figure 3C), with a K_i of 105 mM (Figure 3D).

Uptake of Mannose by HL-60 Cells. Two components were observed when the time dependency of mannose uptake was measured in HL-60 cells (Figure 4A). The amount of cell-associated radioactivity increased rapidly at short incubation times, and the rate of increase was linear for the first 2 min of incubation. Thereafter, the rate of incorporation decreased, and a second component was observed that was linear for at least 10 min. The amount of cell-associated radioactivity after incubation with mannose for 10 min greatly exceeded the amount expected at equilibrium after transport, indicating that the transported mannose is trapped intracellularly. We interpreted these results as indicating that the initial linear phase of incorporation of radioactivity by the HL-60 cells represented the transport of mannose, with the second, slower linear component representing the intracellular trapping of the recently transported mannose. Thus, trapping is expected to be the rate-limiting step when using uptake assays lasting longer than 2 min, and measuring the rate of uptake with incubation times of less than 2 min gives information about transport.

Dose-response studies using 60 s uptake assays revealed that mannose transport by HL-60 cells approached saturation

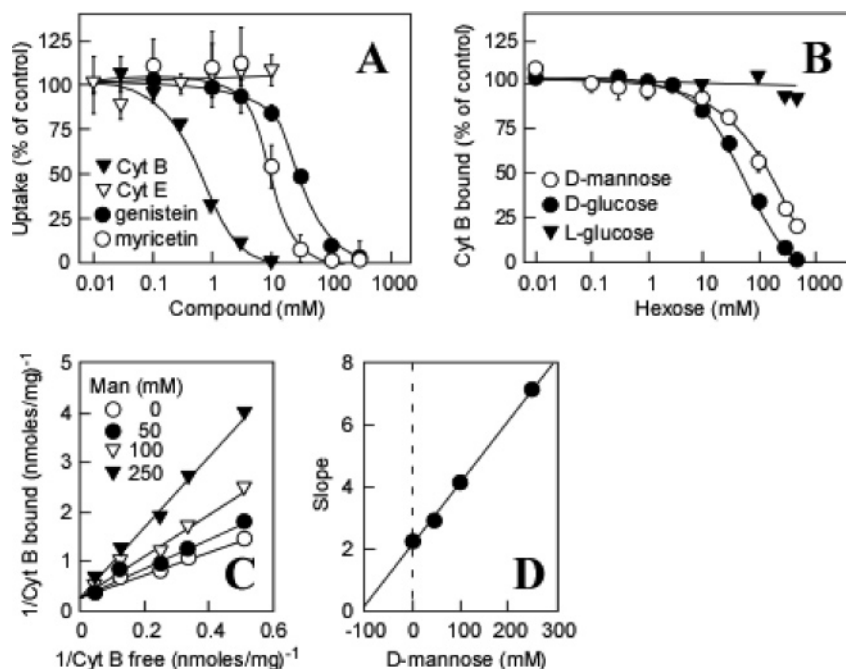


FIGURE 3: Interaction of glucose transporter inhibitors and substrates with the mannose transporter of normal human erythrocytes. (A) Concentration dependence for the inhibition of mannose transport by genistein (●), myricetin (○), cytochalasin B (▼), and cytochalasin E (▽). Measurements were taken using 1 mM mannose and 5 s uptake assays. (B) Concentration dependence for the inhibition of the D-glucose-displaceable binding of cytochalasin B to GLUT1 present in erythrocyte membranes by mannose (○), D-glucose (●), and L-glucose (▼). Measurements were taken using 0.1 μ M cytochalasin B and 10 min binding assays. (C) Double-reciprocal plot of the inhibitory effect of mannose on cytochalasin B binding. Cytochalasin B binding was tested in the absence (○) or in the presence of 50 (●), 100 (▽) or 250 mM (▼) mannose. (D) Secondary plot of the effect of mannose on cytochalasin B binding from the data in panel C. Abbreviations: CytB, cytochalasin B; CytE, cytochalasin E.

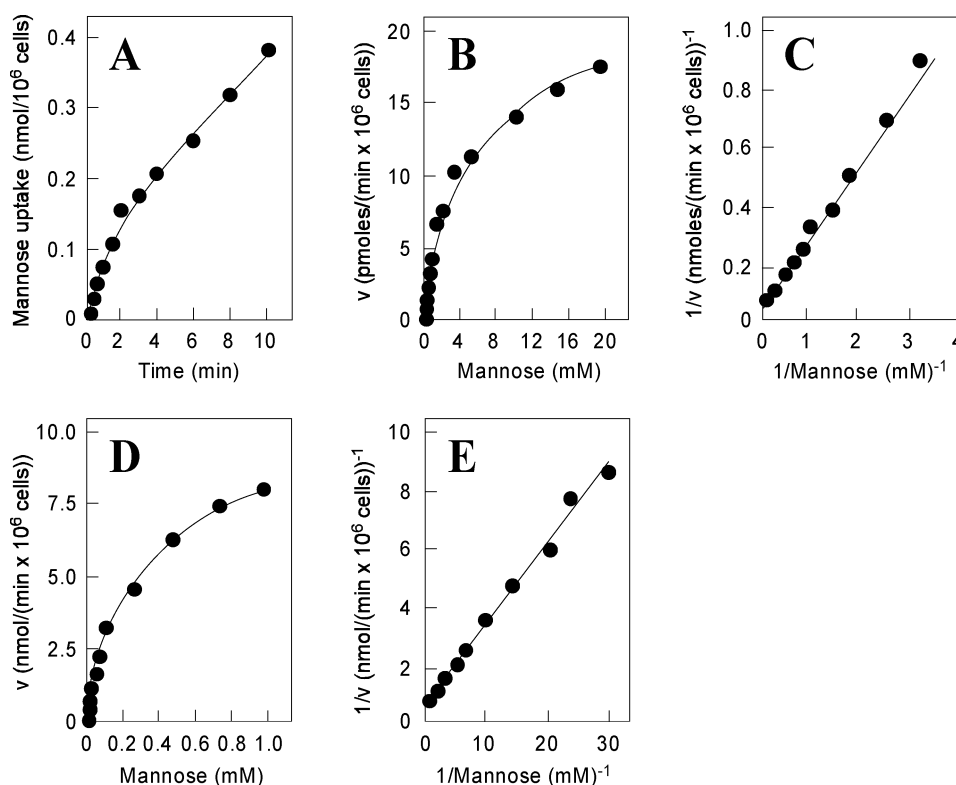


FIGURE 4: Kinetics of the uptake of mannose in HL-60 cells. (A) Time course of uptake of 0.1 mM mannose. (B) Substrate dose dependency of mannose transport using 30 s uptake assays. (C) Double-reciprocal plot of the substrate dose dependence of mannose transport from the data in panel B. (D) Substrate dose dependency of mannose trapping using 10 min uptake assays. (E) Double-reciprocal plot of the substrate dose dependence for mannose trapping from the data in panel D.

at ~ 20 mM (Figure 4B). These studies revealed the presence of a single functional component with an apparent K_m of

6.5 mM and a V_{max} of 4 nmol/min per 10⁶ cells for the transport of mannose (Figure 4C). Dose-response studies

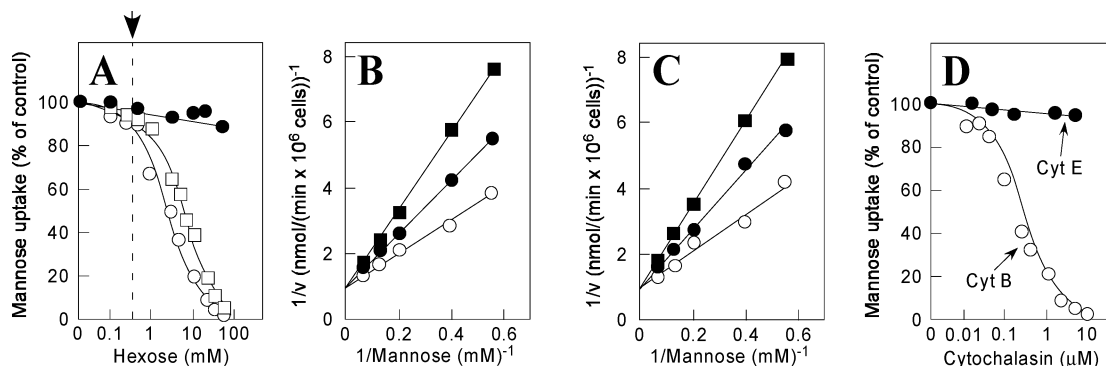


FIGURE 5: Effect of glucose transporter substrates and inhibitors on the transport of mannose in HL-60 cells. (A) Concentration dependence for the inhibition of mannose transport by D-glucose (○), 2-deoxy-D-glucose (□), or L-glucose (●). Experiments were carried out using 0.1 mM mannose and 30 s uptake assays. (B) Double-reciprocal plot of the substrate dependence for mannose transport in the absence (○) or in the presence of 2 (●) or 5 mM (■) D-glucose. (C) Double-reciprocal plot of the substrate dependence for mannose transport in the absence (○) or in the presence of 2 (●) or 5 mM (■) 2-deoxy-D-glucose. (D) Concentration dependence for the inhibition of mannose transport by cytochalasin B (○) or cytochalasin E (●). The arrow at the top and the vertical broken line indicate a concentration of 0.5 mM. Abbreviations: DOG, 2-deoxy-D-glucose; CytB, cytochalasin B; CytE, cytochalasin E.

of trapping using 10 min uptake assays revealed the presence of one component that is saturated at ~ 1 mM mannose (Figure 4D), with an apparent K_m of 0.25 mM and a V_{max} of 1.2 nmol/min per 10^6 cells (Figure 4E). Thus, the kinetic analysis demonstrated the presence of one functional activity involved in the transport of mannose in the HL-60 cells, with a second, higher-affinity component associated with intracellular trapping.

GLUT1 is the facilitative glucose transporter family isoform expressed in the HL-60 cells (9). Therefore, we characterized the dose dependency of the effect of GLUT1 substrates methylglucose, deoxyglucose, and D-glucose, and inhibitor cytochalasin B, on mannose transport by the HL-60 cells using short, 30 s uptake assays. We used a low concentration of mannose in these experiments, 100 μ M, to maximize the possibility of detecting any residual mannose transport activity still functional in the presence of the inhibitors that could represent a high-affinity mannose transporter. The effect of the GLUT1 substrates on mannose transport was dose-dependent; at 0.5 mM, they had only a small effect on mannose uptake, and 50% inhibition was observed at hexose concentrations of 4–5 mM (Figure 5A). At 50 mM, the hexoses inhibited mannose uptake by 100%, and at this same concentration, L-glucose had no effect. Inhibition of transport was competitive (Figure 5B,C), with K_i values of 2 and 3 mM for deoxyglucose and D-glucose, respectively. Cytochalasin B, but not cytochalasin E, inhibited mannose transport with an IC_{50} of approximately 150 nM, and 100% inhibition was observed at 20 μ M cytochalasin B (Figure 5D). The simplest explanation consistent with the data is that there is only one pathway for the transport of mannose in HL-60 cells, and that this pathway corresponds to GLUT1.

We next analyzed the effect of GLUT1 substrates and inhibitors on the trapping of mannose by the HL-60 cells. Competition experiments using 10 min transport assays and 100 μ M mannose indicated that deoxyglucose inhibited, in a dose-dependent manner, the trapping of mannose by the HL-60 cells with an IC_{50} of 3 mM, but no effect of L-glucose was observed (Figure 6A). Total inhibition was observed at deoxyglucose concentrations of ≥ 50 mM under every condition that was tested. Inhibition was mixed noncompetitive, with a K_i of 4 mM (Figure 6B). Cytochalasin B, but not cytochalasin E, inhibited trapping with an IC_{50} of

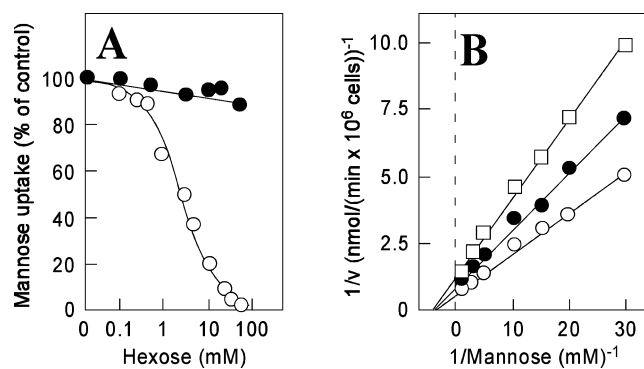


FIGURE 6: Effect of 2-deoxy-D-glucose on the trapping of mannose in HL-60 cells. (A) Concentration dependence for the inhibition of mannose trapping by 2-deoxy-D-glucose (○) or L-glucose (●). Experiments were carried out using 0.1 mM mannose and 10 min uptake assays. (B) Double-reciprocal plot of the substrate dependence for mannose trapping in the absence (○) or in the presence of 2 (●) or 5 mM (□) 2-deoxy-D-glucose.

approximately 200 nM (data not shown). The data are consistent with the concept that the glucose transporter substrates and inhibitors block the cellular trapping of mannose in an indirect manner by interfering with the transport of mannose through the glucose transporters.

In further experiments, we analyzed the presence of a high-affinity mannose transporter in MDCK cells. When the time course of mannose uptake was measured in MDCK cells, the cell-associated radioactivity increased linearly for the first 60 s of incubation, and thereafter, the rate of incorporation decreased to less than 50% of the initial rate (data not shown). Dose–response studies using 45 s uptake assays revealed that mannose transport by MDCK cells approached saturation at ~ 10 mM, with a single component with an apparent K_m of approximately 3 mM. On the other hand, dose–response studies of trapping using 10 min uptake assays revealed the presence of a high-affinity component that is saturated at ~ 0.3 mM mannose, with an apparent K_m of ~ 0.05 mM (data not shown). Thus, the kinetic analysis indicated the presence of a lower-affinity functional component involved in the transport of mannose, with a second, higher-affinity component that can be detected only in longer uptake experiments and therefore associated with intracellular trapping.

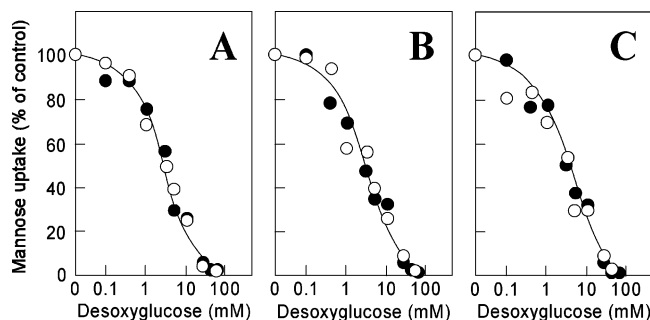


FIGURE 7: Effect of 2-deoxy-D-glucose on the uptake of mannose in human cells. Concentration dependence for the inhibition of mannose uptake by desoxyglucose: (A) normal human mammary epithelial cells (○) and MCF-7 breast tumor cells (●), (B) normal human fibroblasts (○) and normal human neutrophils (●), and (C) U1 leukemia cells (○) and HUVEC endothelial cells (●). Experiments were carried out using 0.1 mM mannose and 60 s uptake assays. Data represent the average of two experiments.

Uptake of Mannose in Human Cells. We analyzed the extent of the participation of glucose transporters in mannose uptake in several cell lines and primary cells of human origin, which included normal breast primary cells, normal fibroblasts, endothelial cells, and breast and leukemia tumor cell lines (Figure 7). Deoxyglucose inhibited mannose uptake in a dose-dependent manner, and at ≥ 50 mM completely blocked the uptake of mannose in all cell types that were tested (Figure 7). The data are consistent with the concept that glucose transporters of the facilitative type are the main route for the entry of mannose in these human cells.

DISCUSSION

A high-affinity component involved in the cellular uptake of mannose was previously identified in experiments measuring the rate of uptake of mannose in various cellular systems (3). The authors of that report advanced the hypothesis that it corresponded to a high-affinity transporter of mannose. We, on the other hand, present data demonstrating that mannose is transported in human cells by facilitative glucose transporters. Moreover, we found no evidence for the existence of a high-affinity mannose transporter that is insensitive to glucose. We used as models for analyzing the cellular uptake of mannose normal human erythrocytes and leukemia cell line HL-60, and extended the findings in these cells to a number of primary cells and cell lines of human origin.

We can easily reconcile the differences between our data and those of Panneerselvam and Freeze (3) as the product of differences in both the experimental methodology and the interpretation of the experimental data. The uptake of substrates by cells is a complex process that couples the transport of the substrate to its intracellular utilization or modification, which in general terms can be viewed as the trapping of the recently transported substrate. Therefore, one consideration when studying the cellular uptake of a substrate is to carry out the experiments using experimental conditions to distinguish the transport of the substrate from its trapping (9). In the specific case of mannose, a trapping step would be its incorporation into the protein. Transport and trapping occur in tandem (with transport occurring before trapping), and they correspond to different processes with their own kinetic properties. Therefore, the rate at which each step

occurs can directly affect the outcome and interpretation of an uptake experiment. When the transport of the substrate is the rate-limiting step of the overall uptake process (transport + trapping), uptake assays for even extended periods of time give information about transport. On the other hand, because in most cases the capacity to transport a substrate exceeds the trapping capacity of the cell, in a typical uptake assay the transport step of the uptake process is very rapid and can be detected only by using short uptake assays; at extended times, the rate-limiting step of uptake is the cellular trapping, and it is no longer possible to assess transport separately and as distinct from trapping. Experimentally, this is manifested in an uptake experiment in which the plot of the rate of uptake (y-axis) as a function of time (x-axis) corresponds to a broken line that shows two slopes representing the rate of the two components: an initial, steeper slope lasting a short period of time representing the rate of transport, followed by a second, slower slope representing the rate of accumulation.

By the above criteria, we observed no evidence of accumulation of mannose in the erythrocytes, which showed a rapid equilibrium of intracellular mannose with the extracellular concentration. Using short uptake assays, we detected only one functional component, with an apparent K_m of 6 mM for the transport of mannose by the erythrocytes. Competition and inhibition experiments using substrates and inhibitors specific for GLUT1 showed that the mannose transporter of the human erythrocytes exhibited the functional characteristics expected for GLUT1, the facilitative glucose transporter expressed in these cells. The following evidence supports this proposal. (1) Mannose transport was completely blocked by methylglucose, deoxyglucose, D-glucose, cytochalasin B, genistein, and myricetin at concentrations at which they block the activity of GLUT1. (2) Control compounds (L-glucose, cytochalasin E, and daidzein) unable to interact with GLUT1 failed to affect mannose transport. (3) Hexoses such as 3-methylglucose and 2-deoxyglucose inhibited mannose transport in a competitive manner. (4) Mannose displaced the glucose-displaceable cytochalasin B bound to erythrocyte membranes in a competitive manner. The simplest interpretation of these data is that GLUT1 is the transporter mediating the entry of mannose in normal human erythrocytes.

Our data indicated that the HL-60 cells express two functional activities involved in the cellular uptake of mannose. One component was detected in short uptake assays measuring transport, and had kinetic and functional properties that are similar to those of the erythrocyte mannose transporter. These similarities included (1) a transport K_m of 6.5 mM, (2) transport being completely inhibited by elevated concentrations of methylglucose, deoxyglucose, D-glucose, and cytochalasin B, and (3) deoxyglucose inhibiting transport in a competitive manner. Thus, our transport and inhibition data strongly support the concept that the mannose transporter present in the HL-60 cells is GLUT1, the facilitative glucose transporter present in these cells. Our data also indicate that mannose transport through the glucose transporters is a mechanism that is not restricted to normal erythrocytes and HL-60 cells and may be a general property common to most human cells. This concept is supported by the results of competition and inhibition experiments that revealed that mannose uptake in different cell types is completely blocked

in the presence of elevated concentrations of deoxyglucose, a substrate specific for glucose transporters of the facilitative type.

A second, higher-affinity component involved in the uptake of mannose in the HL-60 cells was detected only in long uptake experiments designed to assess the intracellular trapping of mannose. We failed to detect this high-affinity activity in short uptake assays assessing transport, using a wide range of mannose concentrations, from 10 μ M to 20 mM. Moreover, the competition experiments using 10 min assays revealed that although deoxyglucose completely blocked mannose uptake in the HL-60 cells under these conditions, the inhibition was noncompetitive. We interpreted the data as supporting the concept that the high-affinity component detected in the HL-60 cells is not involved in the transport of mannose but is instead associated with its intracellular trapping. The noncompetitive inhibitory effect of deoxyglucose can be explained if the hexose is inhibiting directly the primary event responsible for the entry of mannose into the cells, affecting trapping in an only indirect manner (9).

Our data with the HL-60 cells are very similar to those described by Panneerselvam and Freeze (3, 4). They also observed two different uptake rates when assessing the time-dependent uptake of mannose, with the rate of the first component being higher than the second. However, they derived the kinetic constants for mannose uptake from long uptake experiments (from 10 to 60 min), raising the question of the identity of the rate-limiting step under those conditions. The lack of an effect of 0.5 mM glucose on the uptake of low concentrations of mannose was expected on the basis of the known K_m for the transport of glucose by the glucose transporters. These same considerations apply to the issue of the role of the glucose transporters on the cellular acquisition of mannose at the micromolar concentrations observed in plasma; a physiological concentration of sugar will block only partially the transport of mannose through the glucose transporters.

Taken together, our data and those of Panneerselvam and Freeze reveal that the transport of mannose is kinetically separable from its intracellular trapping. A reanalysis of the data of Panneerselvam and Freeze indicates that the high-affinity component observed in extended uptake experiments was mistakenly identified as a high-affinity glucose-insensitive mannose transporter and is instead associated with the intracellular trapping of mannose.

The same reflection applies to the results described in a recent report by Shang and Lehrman (7), which described that metformin incubation of dermal fibroblasts for 24 h induced a 1.8-fold increase in the rate of uptake of mannose. However, these authors measured the rate of mannose uptake via uptake assays typically lasting 10 min at 37 °C. Therefore, their identification of a high-affinity and specific metformin-stimulated D-mannose transporter in fibroblasts should instead be associated with an effect of metformin on the intracellular trapping of mannose. It should be noted that while metformin inhibits uptake of nonmetabolized sugars 2-deoxyglucose and 3-O-methylglucose and therefore is not incorporated into proteins and lipids, there is a stimulation of mannose uptake, which of course is metabolized. This finding is entirely compatible with metformin stimulating mannose metabolism without altering hexose transport.

We conclude that the transport of mannose in human normal erythrocytes and leukemia HL-60 cells occurs through a facilitated mechanism and is mediated by GLUT1, the facilitative hexose transporter expressed in these cells. Our data do not support the existence of a novel, high-affinity glucose-insensitive mannose transporter in these cells. We propose instead that mannose transport through the glucose transporters is the main mechanism for the cellular acquisition of mannose by human cells. The physiological relevance of this transport system is indicated by the fact that it is still capable of transporting mannose in the presence of physiological concentrations of glucose.

A number of reports described the existence of high-affinity sodium-dependent mannose transporters in renal and intestinal epithelial cells, but there is no evidence for the expression of these transporters in other tissues and cells (8, 17–19). These transport systems are low-capacity and high-affinity systems functionally similar to the sodium-dependent glucose transporters, whose expression is restricted to the tissues mentioned above. Thus, functionally as well as in terms of their tissue specific expression, the intestinal and renal mannose transporters are clearly different from the facilitative glucose transporters and are unrelated to the putative glucose-insensitive mannose transporter proposed by Panneerselvam and Freeze and by Shang and Lehrman.

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