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Characterization of glucose transport system in *Drosophila* Kc cells

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We have characterized the glucose transport system in the Kc cells of *Drosophila melanogaster*. The transporter is stereospecific and can be inhibited by both cytochalasin B and phloretin, with k_i values of 4 μM and 30 μM for cytochalasin B and phloretin, respectively. Moreover, phloridzin does not affect uptake of glucose by Kc cells. Thus, on the basis of inhibitor studies, it appears that this insect cell line contains glucose transporters similar to those of the mammalian cells. However, probes prepared from rat transporter cDNAs showed no cross-hybridization with *Drosophila* RNA under moderately stringent conditions. Sugar selectivity has also been investigated by competition assay. The k_m values for D-glucose, 2-deoxy-D-glucose, D-fructose and D-galactose are 2 mM, 6 mM, 17 mM and 16 mM, respectively. The results indicate that this transporter prefers D-glucose and that it does not discriminate between D-galactose and D-fructose.

Glucose transporter; *Drosophila melanogaster*; Kc cell

1. INTRODUCTION

Glucose is one of the primary energy sources for animal cells. In most cases, the translocation of glucose across the plasma membrane is mediated by carrier proteins known as glucose transporter (for a review, see [1]). Results obtained by molecular cloning have suggested that there are at least five different isozymes of glucose transporters (GLUT 1–5) in mammalian cells [2–12]. However, it was demonstrated recently that GLUT 5 is, in fact, a fructose transporter instead [13]. Biochemical and physiological studies also indicate that each type of these transporters displays unique properties, presumably adapted to its specific tissue requirements (for a review, see [14]).

Several genes in *Saccharomyces cerevisiae*, including SNF3 [15], GAL2 [16], HXT1 [17], HXT2 [18], MAL61 [19] and LAC12 [20], have been identified as sugar transporters. While the substrate specificity for these transporters are distinct, they share some sequence homology among one another and to mammalian glucose transporters [15–20]. Since the characteristics of sugar transporters in other phyla are largely unknown, we therefore undertook to characterize the uptake of glucose by *Drosophila* Kc cells to obtain information on insect glucose transport system. Herein, we demonstrate that there is a cytochalasin B-inhibitable glucose transporter in Kc cells, but its substrate selectivity differs from those of mammalian and yeast cells.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

[^{3}H]-2-Deoxy-D-glucose (specific activity 26.2 Ci/mmol) was purchased from DuPont-New England Nuclear. D-Glucose, 2-deoxy-D-glucose and D-fructose were obtained from Merck; and D-galactose, L-glucose, cytochalasin B, phloretin, phloridzin and mineral oil (density 0.875–0.885) were from Sigma. Silicone oil (density 1.050) was purchased from Aldrich.

2.2. *[^{3}H]2-Deoxy-D-glucose uptake assays*

Kc cells of *D. melanogaster* were grown in D-22 culture medium [21] at 25°C for 4–5 days. To perform the uptake assays, the Kc cells were harvested by centrifugation (500 × *g*, 3 min), and resuspended in buffer A (50 mM KH_2PO_4, 60 mM Na_2HPO_4, 50 mM NaCl, 100 mM KCl, 5 mM $MgSO_4$, 0.2 mM D-glucose, pH 7.4). The uptake was initiated by adding [^{3}H]2-deoxy-D-glucose to the cell suspensions to a final concentration of 10 nM (0.5 μCi/ml) and proceeded at room temperature. At specific time intervals, aliquots of the cell suspension were withdrawn and layered over oil mixture (density 1.033 g/ml, by mixing 9 vols. of silicone oil with 1 vol. of mineral oil) in 1.5-ml microfuge tubes. The uptake was terminated by centrifugation through the oil at 16,000 × *g* for 30 s. The pellets were then solubilized with 0.1 ml of 1% SDS in 75 mM NH_4HCO_3, and counted in 3 ml of Aquasol-2 liquid scintillation fluid (DuPont-NEN) with a liquid scintillation counter. When applicable, ^{3}H radioactivity associated with the cell pellets in the presence of 20 μM of cytochalasin B was used to correct for non-specific entry and/or extracellular trapping.

To examine the effects of cytochalasin B, phloretin, and phloridzin, Kc cells resuspended in buffer A were preincubated with varying amounts of these inhibitors for 5 min at room temperature before [^{3}H]2-deoxy-D-glucose was added. After they were incubated for an additional 10 min, the uptake was terminated by centrifugation through the oil and determined as described above.

2.3. *Northern blot analysis*

Total RNA was extracted from whole *Drosophila* or rat tissues by the method of Chirgwin et al. [22]. 50 μg of total RNA was denatured with formamide and formaldehyde and resolved by 1% agarose gel electrophoresis. Then, it was transferred onto nylon membrane (Nytran, Schleicher & Schuell) and hybridized with probes of full-

length cDNAs of rat GLUT 1, 2 and 4 in 50% formamide, 5 × SSPE, 2 × Denhardt's solution and 0.1% SDS at 42°C for 24 h. The probes were labeled with [α-^{32}P]ATP by the random priming method.

2.4. *Determination of sugar affinity to the glucose transporter*

The affinity of sugars, including D-glucose, 2-deoxy-D-glucose, L-glucose, D-galactose, and D-fructose, to the glucose transport system of Kc cells was measured by competition assay. Briefly, Kc cells in buffer A without D-glucose were incubated simultaneously with different concentrations of the specified sugar and 16 nM of [^{3}H]2-deoxy-D-glucose for 10 min at room temperature. Then, uptake was terminated by centrifugation through oil, and the inhibition of [^{3}H]2-deoxy-D-glucose uptake by sugar competitors was quantified. The data were then analyzed as described previously [23].

3. RESULTS AND DISCUSSION

Biochemical and physiological studies on glucose transporters in vertebrates are documented, but the transporters in most of the other phyla are less well characterized. Here, we used *Drosophila* Kc cells as a model to investigate the glucose transport system in insects. Fig. 1 shows the time-course of the uptake of [^{3}H]2-deoxy-D-glucose by Kc cells in the presence of 0.2 mM D-glucose. The uptake appears to be linear for 20 min. Moreover, a sizeable 2-deoxy-D-glucose uptake by Kc cells can be inhibited by 20 μM of cytochalasin B, an inhibitor for mammalian glucose transporter of the facilitated diffusion type. On the other hand, a substantial amount of the uptake could not be inhibited by 20 μM of cytochalasin B, especially for early time points. Identical results were obtained using 100 μM cytochalasin B (data not shown). However, by extrapolation, a significant amount of ^{3}H radioactivity is associated with the Kc cell pellet at time = 0 min (Fig. 1). A large portion of the uninhibitable uptake, therefore, may be contributed to by extracellular trapping of [^{3}H]2-deoxy-D-glucose. We further characterize this glucose transport system.

First, we examined the concentration dependence of

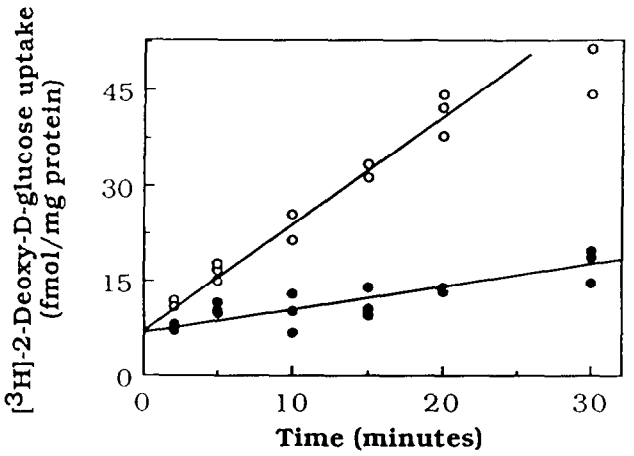

Fig. 1. Time-course of [^{3}H]2-deoxy-D-glucose uptake by *Drosophila* Kc cells. Uptake of [^{3}H]2-deoxy-D-glucose with (●) or without (○) 20 μM cytochalasin B are given here. The amount of protein was determined by Lowry's method.

cytochalasin B on 2-deoxy-D-glucose uptake by Kc cells. As shown in Fig. 2A, the k_i for the cytochalasin B inhibitable component is 4 μM. The value obtained here is comparable to that of GLUT 2 (k_i = 1–2 μM, e.g. see [24,25]) but is one order of magnitude higher than that of GLUT 1 (k_i = 0.1 μM, e.g. see [26]). The effect of phloretin, another inhibitor for glucose transporters in vertebrate cells, on 2-deoxy-D-glucose uptake in Kc cells was also investigated. The results shown in Fig. 2B demonstrate that 80% of the total uptake can be blocked by phloretin with a k_i of 30 μM. It was reported that the k_i values of phloretin for human erythrocytes and rat hepatocytes were 2.4 μM [26] and 40 μM [25], respectively. Based on these inhibitor studies, *Drosophila* Kc cells appear to have glucose transporter of the facilitated diffusion type. We further investigated if phloridzin, an inhibitor for Na^+-dependent glucose

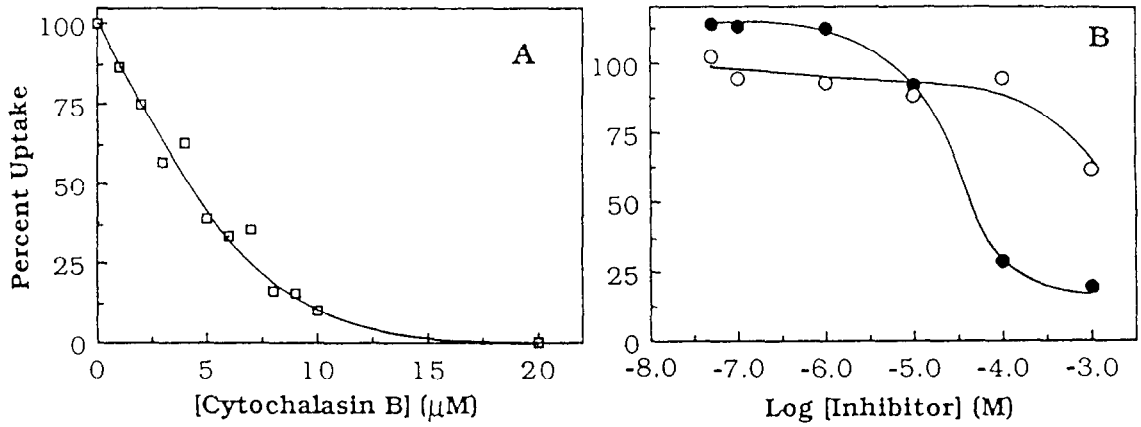

Fig. 2. Effect of inhibitors on [^{3}H]2-deoxy-D-glucose uptake by *Drosophila* Kc cells. (A) Inhibition of [^{3}H]2-deoxy-D-glucose uptake by cytochalasin B is given. The uptake in the presence of 100 μM cytochalasin B is regarded as uninhibitable and is subtracted. (B) Effects of phloretin (●) and phloridzin (○) are shown here. The uptake of [^{3}H]2-deoxy-D-glucose in the absence of inhibitors is taken as 100%; correction on uninhibitable uptake was not made. Each point represents the average of triplicate determinations.

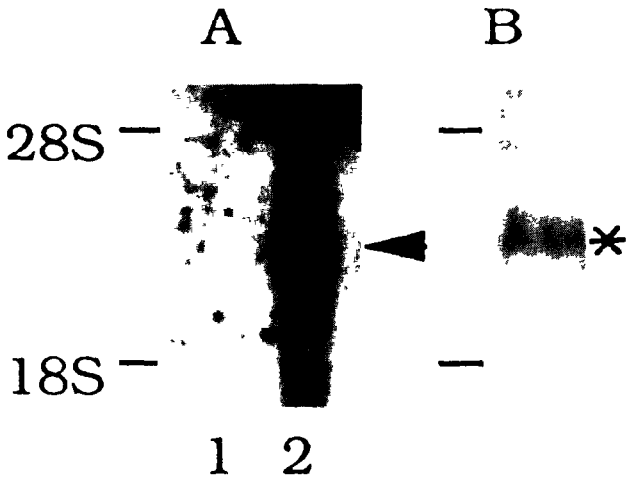

Fig. 3. Northern blot. 50 μg of whole *Drosophila* RNA were displaced by formaldehyde/agarose gel electrophoresis and were transferred on nylon membrane. (A) The blots were hybridized with mixture of rat GLUT 1, 2 and 4 as described in section 2. Lanes 1 and 2 correspond to total RNA of *Drosophila* and rat liver, respectively. The probes react with the 2.9 kb rat liver transporter (arrow head), but fail to recognize any RNA species in *Drosophila*. (B) The blot was also probed with rat hsc70 cDNA. An RNA species of 3.0 kb (asterisk) is recognized by the probes. The positions of 28 S and 18 S rRNA of rat liver are indicated.

transporter of vertebrates, would affect [^{3}H]2-deoxy-D-glucose uptake in Kc cells. It appears that phloridzin has little inhibitory effect at low concentrations (Fig. 2B). However, a high concentration of phloridzin (1 mM) has a moderate effect (Fig. 2B). Since the k_i of phloridzin for the Na^+-dependent transporter is 5 μM [27], it is less likely that such a glucose transport system exists in Kc cells.

Secondly, we asked if the nucleotide sequence encoding the glucose transporter in *Drosophila* is highly homologous to that in mammalians. We isolated RNA from *Drosophila* as well as rat tissues and then carried out Northern blot analysis with full-length cDNAs of rat GLUT 1, 2 and 4 as probes. As shown in Fig. 3A, the probes recognize mRNA of the rat liver transporter (lane 2), but do not hybridize with RNA of *Drosophila* (lane 1). Lack of a cross-reaction is not due to degradation of the RNA, since the probe prepared from rat hsc70 cDNA hybridizes with the homologous gene in *Drosophila* (Fig. 3B). Thus, despite the fact that the transporters in *Drosophila* Kc cells and vertebrate cells can be inhibited to the same extent by cytochalasin B and phloretin, their nucleotide sequences are divergent.

The last question considered in this investigation is the substrate specificity and the influx k_m of glucose for this transport system in Kc cells. The results of a competition assay shown in Fig. 4A indicate that the k_{app} values for D-glucose and D-galactose are 2 mM and 16 mM, respectively. Under the experimental conditions used, the difference between k_{app} and k_m should be negligible. By using identical experimental approaches, the k_m values obtained for 2-deoxy-D-glucose and D-fructose are 6 mM and 17 mM, respectively (Fig. 4B). Thus, the transporter in Kc cells prefers D-glucose over the other hexoses examined; however, it transports D-fructose and D-galactose with equal efficiency. On the other hand, L-glucose shows little effect as a competitor (data not shown), suggesting that this transport system is stereospecific for D-glucose.

The influx k_m of D-glucose for the transporter of *Drosophila* Kc cells is comparable to that for GLUTs of mammalian cells (for a review, see [28]) and to that for SNF3, a high-affinity glucose transporter, of *S. cerevisiae* [29,30]. In contrast, the sugar selectivity for D-galactose and D-fructose of the Kc cell transporter differs from that of mammalian and yeast transporters. We show here that the Kc cell transporter has similar, if not identical, affinity for both D-galactose and D-fructose. Although GLUT 1–3 of mammalian cells are capable of transporting galactose, GLUT 1 and GLUT 3 cannot transport fructose [31] and whereas GLUT 2 can also mediate the uptake of fructose, it is less efficient

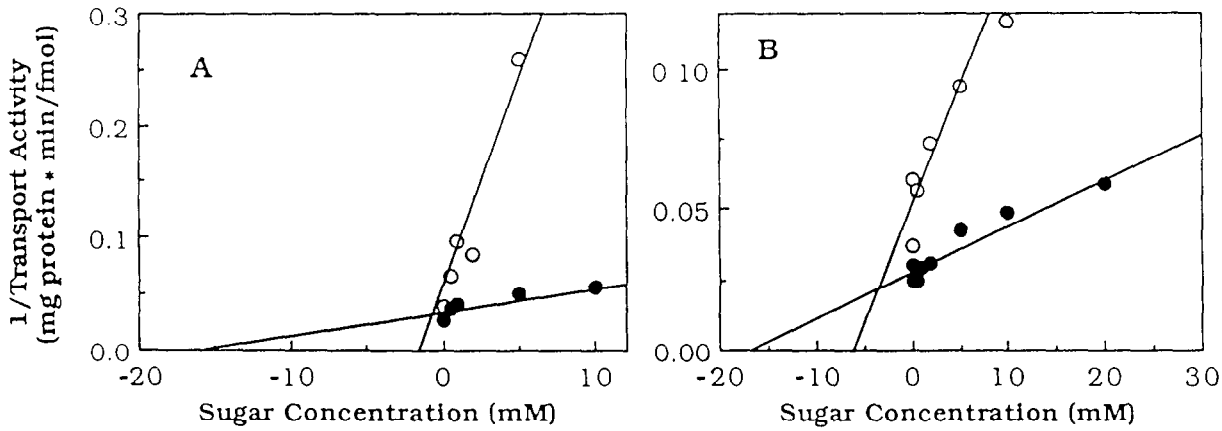

Fig. 4. Competition of [^{3}H]2-deoxy-D-glucose uptake by monosaccharides. (A) D-Glucose (○) and D-galactose (●) were used as competitors. (B) 2-Deoxy-D-glucose (○) and D-fructose (●) were used in the competition assay. Each point represents the mean of triplicate determinations. The intercept on the abscissa is k_{app}.

in the uptake of galactose [31,32]. In contrast, SNF3 has a relatively high affinity for fructose and a low affinity for galactose [29]. Evidently, the relative affinity of galactose to fructose for the transporter of *Drosophila* Kc cells differs from that of mammalian cells or yeasts. The physiological significance in sugar specificity for the *Drosophila* transporter remains unknown and the evolutionary relationship among various glucose transporters still needs to be determined.

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