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HEXOSE TRANSPORT IN PLASMA MEMBRANE VESICLES PREPARED FROM L6 RAT MYOBLASTS

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Hexose transport in plasma membrane vesicles prepared from L6 rat myoblasts was shown to be stereospecific, activated by glucose starvation and occurred by both high and low affinity systems. Transport by the high affinity system was shown to occur by an active transport process. Furthermore, the high affinity system was shown to be defective in vesicles prepared from F72 cells (hexose transport mutant). These results indicate that the high affinity hexose transport system is retained in the plasma membrane vesicles. Thus plasma membrane vesicles could be of value in further characterization of the L6 high affinity hexose transport system, without interference from the various metabolic events occurring in whole cells.

The hexose transport system of the L6 muscle cell line has been the subject of recent investigations (1-6). Hexose transport, not phosphorylation, was found to be the rate-limiting step of hexose uptake in L6 myoblasts (1,4). Furthermore, our results suggest that two hexose transport systems may be operating in these cells (4-6). 2-Deoxyglucose was transported by both high and low affinity systems, whereas 3-O-methylglucose was transported predominantly by the low affinity system. The approaches used to examine the mechanism and regulation of hexose transport in L6 cells has recently been reviewed (7).

Although transport studies with intact cells provide valuable information on the properties of the hexose transport system, interpretation of the results may be complicated by events such as the possible involvement of ATP and phosphorylation, recruitment of cytosolic transport components, posttranslational modification and turnover of transporters, etc. In order to study hexose transport in the absence of these events, we have recently described a method to purify plasma membrane vesicles from L6 cells (8). These vesicles are devoid of ATP, hexokinase, and marker enzymes for cytosol, mitochondria and microsomes.

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In addition, these sealed right-side out vesicles were able to transport D-glucose and 2-deoxyglucose against a concentration gradient, whereas L-glucose only equilibrated across the membrane (8). In this study, we report further characterization of the hexose transport systems in plasma membrane vesicles.

MATERIALS AND METHODS

Materials: 2-Deoxy-D-[^{3}H(G)]-glucose (8.3 Ci/mmol), 3-O-[methyl-^{3}H]-methyl-D-glucose (60-90 Ci/mmol) and D-[5,6-^{3}H]-glucose (78 Ci/mmol) were purchased from New England Nuclear (Boston, MA). 2-Deoxy-2-fluoro-D-glucose was purchased from Calbiochem (La Jolla, CA). All other chemicals were from commercial sources and were of the highest available purity.

Cell Lines and Culture Media: Yaffe's L6 rat skeletal myoblast line (9) was maintained in Alpha medium supplemented with 10% horse serum and 50 ug/ml of gentamycin as previously described (2). In experiments in which cells were grown in glucose-free conditions, the same medium was prepared without glucose and supplemented with dialyzed horse serum and 0.1% fructose. The hexose transport mutant, F72, was maintained on fructose medium supplemented with 0.05 mM 2-deoxy-2-fluoro-D-glucose as described previously (6).

Transport in Plasma Membrane Vesicles: Plasma membrane vesicles were isolated and purified from L6 cells by a previously described method (8), which separated sealed right-side out plasma membrane vesicles (fraction A) from leaky membrane sheets or inside-out plasma membranes (fraction B). Transport assays were determined by the flow dialysis method, transport rates were indicated by the difference between hexose associated with fraction A and with fraction B (8).

Determination of protein: Protein determinations were made by the method of Lowry *et al* (10), using bovine serum albumin as a standard.

RESULTS

Having isolated plasma membrane vesicles from L6 cells, it was important to determine whether these vesicles have similar hexose transport properties as whole cells. Table 1 shows the effect of various hexose analogues on the transport of 2-deoxyglucose. It can be seen that L-glucose, D-fructose, D-glucosamine and 3-O-methylglucose, at the concentrations used, have no effect on 2-deoxyglucose transport. On the other hand, D-glucose, 2-deoxy-2-fluoro-D-glucose and D-galactose significantly reduced 2-deoxyglucose transport activity. These results are similar to those observed with whole cells (4) and indicate that the vesicles possess a specific carrier-mediated hexose transport system. Table 1 also shows that phloretin and cytochalasin B are good inhibitors of transport activity. These reagents are potent inhibitors of carrier-mediated hexose transport, whereas phlorizin is not (4).

TABLE 1

Inhibition of 2-Deoxyglucose Transport by Glucose Analogues

Inhibitor (3 mM)	Rate of Transport* (nmol/min/mg protein)	% of Control
Control	354	100
L-Glucose	369	104
D-Glucose	98	28
D-Fructose	416	118
D-Glucosamine	405	114
3-O-Methylglucose	335	95
D-Galactose	211	60
2-Deoxy-2-fluoro-D-glucose (0.5 mM)	229	65
Cytochalasin B (5 uM)	196	55
Phloretin (20 uM)	143	40
Phlorizin (20 uM)	339	96

*The concentration of 2-deoxyglucose used was 500 uM with a specific activity of 16 mCi/mmol. The concentration of vesicles used was 50 ug protein/ml. Results are the average of at least 4 trials.

Glucose starvation has been shown to activate hexose transport in L6 cells (4-6), as well as in other cell lines (11-14). This increased rate of hexose transport is due to an increase in the number of hexose carriers operating in the plasma membrane and was found to require protein synthesis (4,12,15). To determine if this transport property is maintained in the vesicles, transport assays were performed in vesicles prepared from glucose-grown and glucose-starved (fructose-grown) L6 cells. Figure 1 demonstrates that an increase in transport activity is observed in vesicles prepared from glucose-starved cells.

We have previously demonstrated that 2-deoxyglucose is transported in L6 cells by both high and low affinity systems (apparent K_m of 0.6 mM and 3.0 mM, respectively) (4). On the other hand, 3-O-methylglucose is transported predominantly by the low affinity system (apparent K_m of 3.5 mM) (4). Figure 2 indicates that similar kinetic properties are also observed in the vesicles. As demonstrated in Figure 2A, 2-deoxyglucose is transported by a high affinity system (apparent K_m of 0.6 mM) and a low affinity system with an apparent K_m approaching infinity, indicating passive diffusion. This would suggest that the low affinity carrier system is not operating in the vesicles and may have resulted from loss or inactivation of some component(s) during vesicle

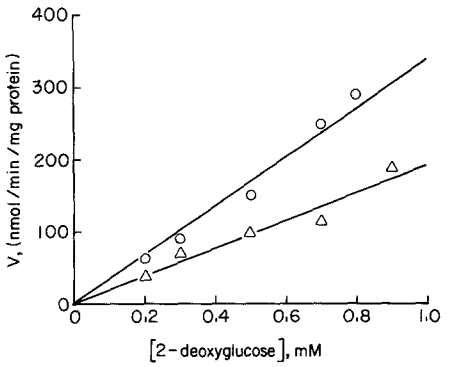


FIGURE 1. The Effect of Glucose Starvation on 2-Deoxyglucose Transport. The transport of various concentrations of 2-deoxyglucose (specific activity of 16 mCi/mmol) were performed in vesicles prepared from glucose-grown (Δ) and glucose-starved (fructose-grown) (O) L6 cells. The concentration of vesicles used were 50 ug protein/ml. The results the average of at least 3 trials.

preparation. This is supported by Figure 2B which shows that 3-O-methylglucose is taken up by passive diffusion. The loss of the low affinity system is not due to lack of substrate phosphorylation since 3-O-methylglucose is not phosphorylated in whole cells (4).

We have previously isolated a mutant, F72, which is defective in the high affinity hexose transport system (6). Figure 3 shows that hexose transport in F72 occurs only by passive diffusion; again showing that the low affinity transport system is not functioning in the vesicles. More importantly, this

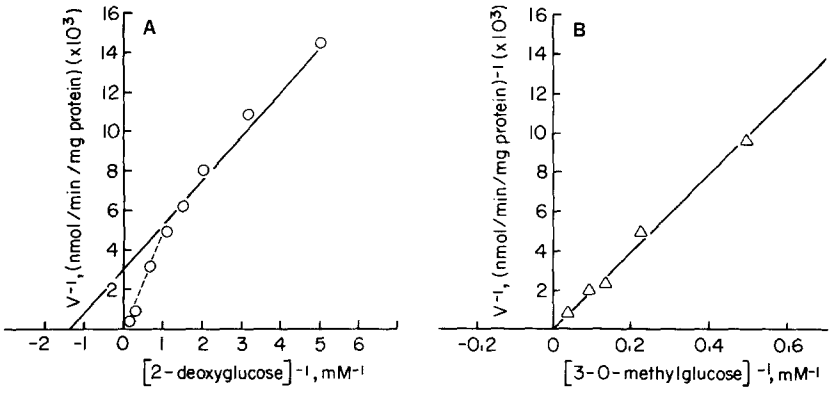
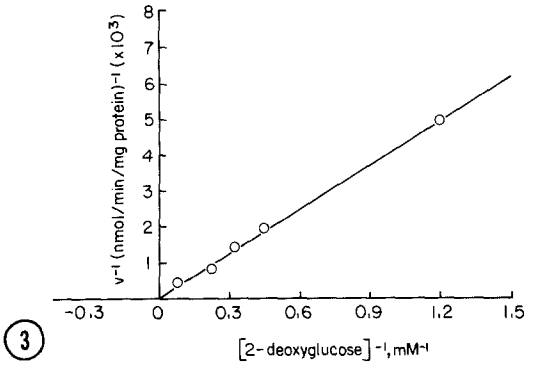


FIGURE 2. Hexose Tranpsort Properties of Plasma Membrane Vesicles. Double reciprocal plots of 2-deoxyglucose (A) and 3-O-methylglucose (B) transport respectively. Studies were carried out with vesicles prepared from fructose-grown cells (50 ug protein/ml). The results are the average of at least 3 trials.



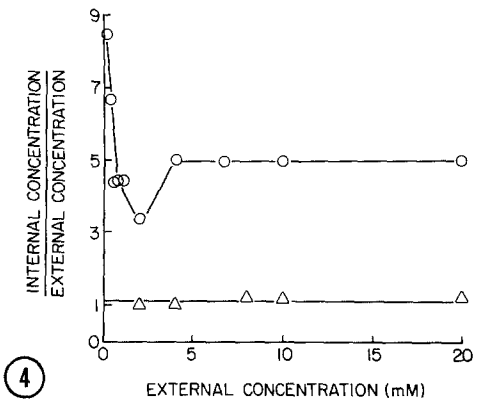


FIGURE 3. 2-Deoxyglucose Transport in Mutant F72. The transport of various concentrations of 2-deoxyglucose were performed in vesicles prepared from F72 cells. The concentration of vesicles used were 35 ug/ml. The results are the average of 4 trials.

FIGURE 4. Intravesicular Accumulation of Hexoses. The intravesicular concentration of 2-deoxyglucose (O) and 3-O-methylglucose (Δ) were determined and compared to the external concentrations at the end of the transport assays. Vesicles were prepared from fructose-grown cells (50 ug protein/ml). The results are the average of at least 4 trials.

study shows that F72 vesicles is defective in the high affinity system, which is present in the L6 vesicles.

Transport studies with whole cells indicated that the high affinity hexose transport system is an active transport process, whereas the low affinity system transports hexoses by facilitated diffusion (4). In order to determine whether this is the case with membrane vesicles, the intravesicular accumulation of various sugar analogues were determined. Figure 4 shows that the intravesicular concentration of 2-deoxyglucose is at least five to ten times higher than the external concentration, regardless of the external concentrations used. We have previously demonstrated that 2-deoxyglucose is not phosphorylated upon entrance into the vesicles (8). This suggests that 2-deoxyglucose is accumulated against a concentration gradient. Similar results were observed when D-glucose was used as a substrate (8). Figure 4 also shows that 3-O-methylglucose only equilibrates across the plasma membrane.

In agreement with the above observations, whole cell transport studies show that the high affinity system, but not the low affinity system, can be inactivated by various energy uncouplers and ionophores (4). Table 2 shows that

TABLE 2

Effect of Ionophores and Energy Inhibitors on Hexose Transport in Membrane Vesicles

Inhibitor	Rate of Transport* (nmol/min/mg protein)	% of Control
1) Control	2994	100
Valinomycin (5 ug/ml) + KCl (10mM)	2831	95
2) Control	339	100
Valinomycin (5 ug/ml) + KCl (10mM)	74	22
CCCP (50 uM)	195	58
DNP (1 mM)	128	38

*Transport substrates used were 1) 5 mM 3-O-methylglucose and 2) 0.5 mM 2-deoxyglucose with specific activities of 1.6 mCi/mmol and 16 mCi/mmol, respectively. Membrane vesicles (25 ug protein) were used immediately after mixing with the inhibitors. The results are the average of at least 3 trials.
CCCP - carbonyl cyanide m-chlorophenyl hydrazone
DNP - 2,4-dinitophenol

these reagents also inhibit 2-deoxyglucose, but not 3-O-methylglucose, transport into vesicles. This supports the contention that the high affinity system may be an active transport process (4). It is presently not clear, though, on the mechanism which provides the energy driving this active transport in the vesicles. We are currently investigating this problem.

DISCUSSION

The purpose of this investigation was to demonstrate that purified plasma membrane vesicles prepared form L6 rat myoblasts could also be used to study the properties of the hexose transport systems. Studying hexose transport in vesicles would be advantageous since the interpretation of the results are not complicated by the many metabolic events. Results from this study indicate that the plasma membrane vesicles retain the high affinity hexose transport system found in whole cells; whereas the low affinity system appears to be nonfunctional in the vesicles. These can be summarized as follows. (i) Hexose transport is stereospecifically inhibited by various hexose analogues. (ii) Hexose transport rates are about 2-fold higher in vesicles prepared from glucose-starved when compared to glucose-grown L6. (iii) Kinetic studies

indicate that the high affinity hexose transport system has an apparent K_m value similar to that observed in whole cells; whereas 3-O-methylglucose is taken up only by passive diffusion. (iv) Vesicles prepared from a high affinity hexose transport mutant are also defective in the high affinity system. (v) The high affinity system may be an active transport process since 2-deoxyglucose is transported against a concentration gradient into the vesicles.

It may be apparent from this study that plasma membrane vesicles can serve as a useful tool in studying transport processes in the absence of cellular metabolism. We have recently used plasma membrane vesicles to examine the mechanism by which anti-L6 antibody can stimulate hexose transport (16).

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