THE EXISTENCE OF TWO MEMBRANE TRANSPORT SYSTEMS FOR GLUCOSE IN SUSPENSIONS OF SUGARCANE CELLS[1,2]

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SUMMARY

Transport of glucose in sugarcane cells depends upon the prior history of cell incubation. High concentrations of glucose suppress a transport mechanism characterized by high affinity for the cells, insensitivity to the presence of F^-, and saturation of the system at 100-200 μM exogenous glucose. This system (System A) becomes expressed when glucose concentration in the medium is kept below 0.1% (5.6 mM). System B is characteristic of cells grown in high glucose concentrations. It has low affinity for the cells, is inhibited by F^-, and saturates at about 1 mM exogenous glucose concentration. Repression of System A occurs in a few minutes while its derepression requires about 7 hr. Cycloheximide and anisomycin inhibit derepression of System A.

The existence of two distinctly different systems for glucose transport has been demonstrated in *Neurospora crassa* (1,2,3,4). Kinetically the two transport mechanisms show affinities for binding sites differing by a factor of approximately 10^3 and they have divergent physiological characteristics. The high K_m system was designated as System I and the low K_m system as System II (3).

We previously reported on a glucose transport system functioning in suspension cultures of sugarcane cells (5). For that investigation all cell

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cultures were prepared in a medium containing 2% (56 mM) sucrose. Under these conditions a low K_m system was the predominant mechanism by which glucose was taken up by the cells. Subsequently it was discovered that pretreatment of the cells in a medium in which glucose replaced sucrose caused glucose to be transported by a system with a higher K_m and a higher level of saturation. The present paper describes features of these glucose transport systems which resemble the two systems described for *Neurospora*.

MATERIALS AND METHODS

Uniformly labeled ^{14}C-glucose (207 mCi/mM) was purchased from New England Nuclear Corporation.

Cells of sugarcane originated from parenchyma tissue of a Hawaiian clone, 50-7209, and were subcultured monthly in a medium containing yeast extract (YE) (6). For kinetic studies, 30 ml of cells were transferred to a YE medium containing the appropriate sugar and were incubated for 1 week. The cells were transferred to 100 ml of a modified White's basal medium in the presence of the sugar for 60 hr, and finally, were transferred to a similar volume of the basal medium lacking the sugar for 5 hr.

For uptake measurements 20 ml of cells were incubated at 22°C with continuous stirring in 50 ml of fresh basal medium under the conditions described for each experiment. Cell samples were removed with a pipet at 30-sec intervals, washed with cold water on a filter, and immediately frozen. The lyophylized cells were digested with Soluene 100 (Packard Instrument Co.) for 60 min at 37°C. Ten ml of scintillation fluid were added and samples were counted by scintillation spectrometry.

RESULTS AND DISCUSSION

Table I shows the K_m values of two systems, designated as Systems A and B, which differ by a factor of 10^2. While K_m values are reproducible, there is some variation in maximum velocities from one experiment to another. Sys-

Table I. Comparison of K_m values and Vmax obtained from transport curves for the transport of glucose by Systems A and B in sugarcane cells.

	K_m mM	Vmax nmoles/mg/min
System A	0.02	0.91
System B	1.40	0.45

Table II. Inhibition of glucose uptake via Systems A and B in sugarcane cells by dinitrophenol (10^{-4}M), azide (10^{-3}M), and fluoride (10^{-2}M).

	Percent inhibition (compared to control)		
	Dinitrophenol	Azide	Fluoride
System A	92.1	77.2	0
System B	91.4	89.6	44.0

tem A further differs from System B in that it is not inhibited by F^- (Table II). Both systems are inhibited by azide and 2,4-dinitrophenol and are, therefore, energy-dependent.

Table III shows the uptake of glucose as concentrations of glucose and sucrose in the medium are changed. Growth in the complete absence of carbohydrate or in the presence of sucrose as the carbohydrate source, caused the predominance of System A. In a mixture of the two sugars, even at a relatively low concentration of glucose compared to that of sucrose, System A remained suppressed. Reducing sugar concentrations in the medium contributed by hydrolysis of sucrose were very low.

When cells were cultured on 2% (112 mM) glucose, then transferred to a

Table III. Glucose uptake by sugarcane cells, after the cells had been grown in the presence of varying concentrations of either glucose or sucrose, or a combination of the two sugars for a period of 7 days. The concentration of glucose in the uptake media was 5 μc ^{14}C-glucose and 5 μM carrier.

Conditions prior to uptake		Glucose uptake
Glucose (mM)	Sucrose (mM)	(nmoles/mg dry cells/min)
0	0	0.320
0	14	0.270
0	28	0.220
0	42	0.130
0	56	0.150
28	0	0.016
56	0	0.002
60	0	0.001
112	0	0.001
17	48	0.015
39	36	0.002
56	28	0.001

medium containing 2% (56 mM) sucrose, reappearance of System A occurred very slowly. System A had not reached a maximum value after 72 hr. However, when cells were conditioned on sucrose for 7 days, and then transferred to glucose for a 12-hr period prior to complete withdrawal of sugars from the medium, reversal from System B to System A was substantially complete after 7-8 hr (Fig. 1). There appeared to be a 2-hr lag before a more rapid recovery rate of System A was established. The reverse process, repression of System A, occurred within a few minutes after adding 2% glucose. The exact time required for repression is difficult to ascertain, since uptake measurements involve 4 min. We know that System A transports 3-O-methylglucose readily (5)

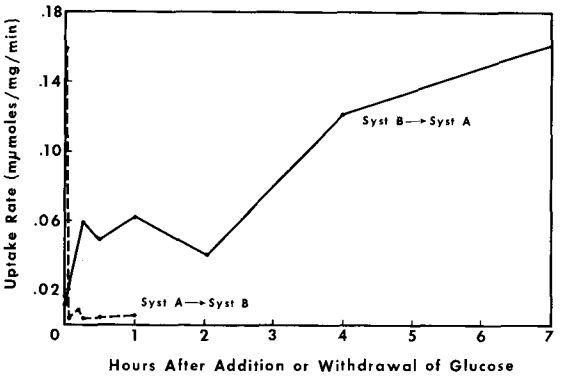

Fig. 1. Time course of reversal of System A to System B and of System B to System A in sugarcane cells after removal of cells from media containing either glucose or no sugars. To induce System A cells were grown on 2% sucrose for 7 days until start of reversion to System B was initiated by withdrawal of sugars from the medium. To cause the predominance of System B cells were grown on 2% sucrose for 6 days and were transferred to medium containing 2% glucose 12 hours prior to initiating reversion. All uptake rates were obtained with cells incubated in a medium containing 5 μc ^{14}C-glucose and 5 μM glucose carrier. Zero time measurements were made immediately prior to transfer of cells to the reversal medium.

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but although the analog avoids possible complications arising from the use of glucose and its subsequent metabolism we decided against using 3-O-methyl-glucose for these experiments. The transport of 3-O-methylglucose by System B was almost undetectable, and we were interested in a direct comparison of repression and derepression.

Schneider and Wiley (4) report a $T_{\frac{1}{2}}$ of 40 min for repression of their System II (equivalent to our System A). This is an appreciably longer period of time than we were able to demonstrate. Thus it is doubtful that the decay of mRNA as described for the *Neurospora* system (7) is functional in sugarcane cells. The inhibitory effect of cycloheximide and anisomycin on derepression (Fig. 2) suggests, however, that translational control may be important for System A to become expressed. With repressed cells the reduction in uptake rate of cell cultures containing these two antibiotics over a control containing neither was 72%, whereas a similar reduction of uptake rates in derepressed cells was more than 94%. Therefore, cycloheximide and anisomycin

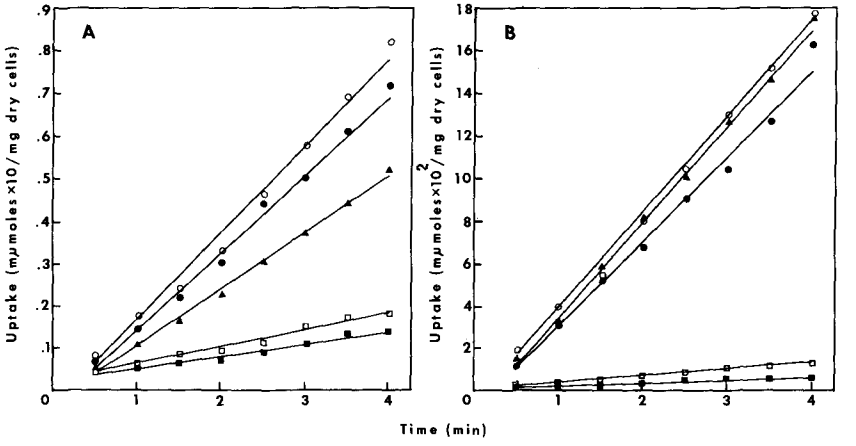

Fig. 2. The effect of antibiotics on the uptake of glucose by sugarcane cells. The cells were incubated on medium containing 2% glucose for 7 days. Twenty ml aliquots of the cell suspension were transferred to 50 ml of basal medium containing either 2% glucose (A) or 2% sucrose (B) and cycloheximide; (1 μmole) ■——■, anisomycin (5 μmoles) □——□, actinomycin D (0.16 μmoles) ●——● or puromycin (5 μmoles) ○——○. A control without antibiotic, ▲——▲, was included for each (A) and (B). After 60 hr the cells were transferred to basal medium without sugars and without antibiotics for an additional 5 hr prior to ^{14}C-glucose (5 μc) uptake measurements, using 5 μM glucose carrier.

each reduced derepression by about 22%. There was no effect of puromycin or actinomycin D on derepression but it is not known whether these antibiotics can penetrate the cell membrane.

The similarity of a dual glucose transport mechanism in fungi and higher plants has been established. In the intact plant, a cell-wall-bound invertase provides the parenchyma cell with adequate exogenous reducing sugars under normal conditions (8). It is difficult to conceive of a physiological condition which could reduce the free-space glucose concentration of intact sugarcane hybrids to a point at which System A is needed for reducing sugars influx. It is possible that this transport system is a vestige of a mechanism required by wild species of sugarcane. Significantly, the kinetics of glucose influx measured in sugarcane parenchyma tissue slices is in close agreement with System B (9).

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