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PROPERTIES OF THE HEXOSE TRANSPORT SYSTEMS OF *ASPERGILLUS NIDULANS*

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SUMMARY

1. Systems for the transport of D-glucose, D-galactose, and D-fructose by young mycelium of *Aspergillus nidulans* were studied. Properties of the glucose system were determined by use of isotopic 2-deoxy-D-glucose, a nonutilizable glucose analog; the uptake of isotopic D-galactose and D-fructose were measured by mutants that could not metabolize these sugars.

2. Systems for the transport of these three sugars, are constitutive and carrier-mediated, since they show saturation kinetics with respect to concentration, and are subject to competitive inhibition.

3. The uptake of all three sugars takes place against a concentration gradient, and is energy dependent.

4. The relationship between structure and affinity for the transport systems was studied by measurement of competitive inhibition exerted by various sugars and their derivatives on the uptake of 2-deoxy-D-[$^{14}C_6$]glucose and D-[$^{14}C_6$]galactose. Sugars could thus be ordered with respect to their affinities for the transport systems.

5. In the case of both the D-glucose and D-galactose systems, no single substituent of the C1 pyranose ring was essential for carrier-binding activity; any single change was tolerated, with the exception of methylation of the anomeric hydroxyl group. Any two changes abolished activity.

6. While D-galactose competes for the D-glucose system and *vice versa*, systems for the transport of these two sugars are distinct.

7. D-Fructose is transported by a highly specific system, since no sugar tested competed with its uptake.

INTRODUCTION

It was observed by ROMANO AND KORNBERG[1,2] that acetate restricts the uptake of certain sugars by *Aspergillus nidulans*, and the hypothesis was formulated that acetyl coenzyme A, as an endproduct of glycolysis, can regulate sugar utilization by controlling sugar uptake. An understanding of this possible regulatory system is dependent upon a better understanding of the mechanism of sugar transport in

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filamentous fungi. And in spite of the significant advances in the elucidation of uptake systems in bacteria[3–5] on the one hand and yeast[6,7] on the other, relatively little is known concerning sugar transport in fungi. BROWN AND ROMANO[8] have shown that the active transport of the glucose analog 2-deoxy-D-glucose by *A. nidulans* does not involve phosphorylation, a situation that is in contrast to that found in many bacteria[5] and in bakers' yeast[9,10]. The present work describes other properties of the hexose transport system of this organism, and deals chiefly with the relationships between sugar structure and capacity for uptake. The relative affinities of various sugars and sugar derivatives for the glucose transport system have been determined by measuring the degree of competitive inhibition that they exert on the transport of 2-deoxy-D-[$^{14}C_6$]glucose, which has been established to be a non-metabolizable substrate of the glucose system. Thus a comparison of K_i values allows a convenient procedure for ordering sugars with respect to their carrier-complexing activity, as has been done by CIRILLO[7] with yeast, LEFEVRE[11] with erythrocytes, ROGERS AND YU[12] with bacteria, and BARNETT *et al.*[13] with mammalian intestine.

MATERIALS AND METHODS

D-[$^{14}C_6$]Glucose, D-[$^{14}C_6$]galactose, D-[$^{14}C_6$]fructose, and 2-deoxy-D-[$^{14}C_6$]-glucose were obtained from International Chemical and Nuclear Corp., Irvine, Calif. Non-radioactive sugars were purchased from Mann Research Laboratories, New York, N.Y., with the exception of 1,5-anhydro-D-glucitol, which was obtained from NK Laboratories, Jersey City, N.J.

Organisms

A. nidulans R46 (pro 1, y, pyro 4), a strain auxotrophic with respect to proline and pyridoxin, and producing yellow conidia, but wild type with respect to sugar utilization was used to measure uptake of the non-metabolizable glucose analog 2-deoxy-D-glucose. The uptake of D-galactose and D-fructose were measured with mutants which were deficient in the ability to metabolize these sugars. Thus, *A. nidulans* R31 (bi 1, w 3, gal 9) a white-spored biotin auxotroph, which was shown by ROBERTS[14] to be galactokinaseless (EC 2.7.1.6), and *A. nidulans* R139 (y 2, pyro 4, fru 1), a yellow spored pyridoxin auxotroph which cannot grow on fructose as the consequence of a lack of fructokinase (EC 2.7.1.4), were used to study the uptake of D-galactose and D-fructose, respectively. All strains were kindly provided by C. F. Roberts, Department of Genetics, University of Leicester, England.

Stock cultures were maintained on a complex medium containing per l distilled water: D-glucose, 20 g; malt extract, 20 g; peptone (Difco), 1 g; agar, 20 g. For uptake experiments, cells were grown in the minimal synthetic medium described by PONTECORVO *et al.*[15], with the addition of carbon sources as specified in the results section, and appropriate amino acid and vitamin supplements. Concentrations of supplements were: proline (5 mg/l) and pyridoxin (50 μg/l) for *A. nidulans* R46, biotin (20 μg/l) for *A. nidulans* R31, and pyridoxin (50 μg/l) for *A. nidulans* R139.

Uptake Experiments

Cells were grown from spores for 18–24 h, depending on the strain and carbon source used, in 400 ml minimal medium contained in a baffled 2-l flask. Flasks were

inoculated with 2 ml of a spore suspension in 0.9% saline containing 0.01% Tween 80 and incubated at 37° on a rotary shaker at 250 rev./min. Cells were harvested by filtration, washed with water, and resuspended in 50 mM phosphate buffer, pH 7.0 in the case of *A. nidulans* R46 and R139, and pH 6.0 in the case of *A. nidulans* R31 (the lower pH was used in the latter case because the mutant characteristic of this strain is better expressed below neutrality[14]). Cell density was adjusted to approximately 1 mg dry wt. per ml, and determined by collecting cells from 20-ml aliquots on dried and tared membrane filters (0.8 μm porosity; Millipore Corp., Bedford, Mass.) and drying to constant weight at 80°.

Isotopic sugars and other additions as indicated in the results section were added to 50 ml of cell suspension contained in a 250 ml flask after 30 min preincubation at 37° on a wrist action shaker. Following addition of isotopic sugar, 5-ml samples were removed at appropriate time intervals, filtered intermediately through membrane filters (0.8 μm porosity) and washed with 15 ml of buffer. The filters with the cells thereon were then transferred to scintillation vials containing 10 ml BRAY'S[16] scintillation fluid for counting in a Packard liquid scintillation spectrometer.

Results expressed in terms of μmoles sugar taken up per unit dry wt. cells are based on a counting efficiency of 85% and specific activity of isotope added, as indicated in the results section. Intracellular concentrations of sugar expressed in terms of molarity are based on a water content of 80%, so that 1 mg dry cells is equivalent to 4 μl intracellular water.

Determination of apparent K_m and K_i values

Values for the apparent K_m for the transport of 2-deoxy-D-glucose, D-galactose, and D-fructose were determined graphically by the method of LINEWEAVER AND BURK[17], as shown in RESULTS. The inhibition of 2-deoxy-D-[$^{14}C_6$]glucose uptake by 1,5-anhydro-D-glucitol, 6-deoxy-D-glucose, D-galactose, D-mannose, and D-xylose, and the inhibition of D-[$^{14}C_6$]galactose uptake by D-fucose, L-arabinose and 2-deoxy-D-glucose all showed kinetics of competitive inhibition in Lineweaver-Burk plots. Thus the following equation could be used to calculate the apparent K_i:

$$K_i = \frac{I}{\frac{K_p}{K_m} - 1}$$

where I represents the concentration of inhibitor, and K_p and K_m are values read graphically as the intersection of the lines with the abcissa in the presence and absence of inhibitor, respectively.

Other sugars were tested as competitors of 2-deoxy-D-[$^{14}C_6$]glucose, D-[$^{14}C_6$]-galactose and D-[$^{14}C_6$]fructose uptake at 10–100 times the concentration of substrate. Apparent K_i values were calculated using the following equation, according to the method used by CIRILLO[7]

$$K_i = \frac{I}{(v_0/v_i)\,(S/K_m + 1) - (S/K_m + 1)}$$

where S and I are the concentrations of substrate and inhitor respectively, v_0 and v_i are the velocities of uptake in the absence and presence of inhibitor, and K_m is the value measured in the absence of inhibitor.

Interpretation of sugar structure

Conventions with regard to the configuration of sugar molecules in solution are as reviewed by CIRILLO[7]. Thus glucose is considered as a C1 glucopyranose chair; other sugars are considered as variations of this structure, and are assumed to be in the C1 configuration unless specified otherwise.

RESULTS AND DISCUSSION

Systems for the transport of D-glucose (as measured by the uptake of 2-deoxy-D-glucose), D-galactose, and D-fructose by *A. nidulans* are all constitutive, as indicated in Fig. 1. For each of these sugars, the rates of uptake by cells grown in the absence or presence of the specific substrate were virtually identical. This is in agreement with the results of ROBERTS[14] with respect to galactose uptake.

These systems must be classified as active transport systems, since the uptake of each of these hexoses is dependent upon energy, and intracellular concentrations of the accumulated sugars exceed that of the external medium. Uptake was strongly inhibited by 2,4-dinitrophenol (Fig. 2). The concentrative nature of the uptake systems is shown in Table I. It is clear that each of these three hexoses was accumulated against a gradient, and while the final intracellular concentration varied directly as the external concentration, the concentration factor was greatest at the lowest external sugar concentration.

The rates of 2-deoxy-D-glucose, D-galactose, and D-fructose transport are concentration dependent and exhibit saturation kinetics characteristic of carrier-mediated transport. Fig. 3 shows Lineweaver–Burk plots for the uptake of these sugars. Apparent K_m values calculated from these data are: D-galactose, $3 \cdot 10^{-5}$ M; D-fructose,

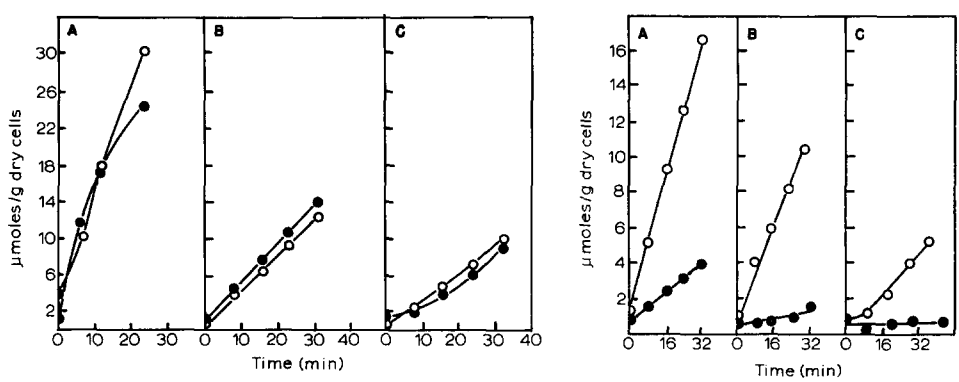


Fig. 1. Uptake of 2-deoxy-D-glucose, D-galactose, and D-fructose by induced and non-induced *A. nidulans* cells. (A) *A. nidulans* R46 grown on 50 mM glucose (○) or 50 mM glycerol (●), incubated with 2-deoxy-D-[$^{14}C_6$]glucose (0.1 mM, 0.2 μC/μmole). (B) *A. nidulans* R31 grown on 50 mM glucose + 50 mM sodium acetate (○) or 50 mM glucose + 50 mM sodium acetate + 50 mM galactose (●), incubated in the presence of D-[$^{14}C_6$]galactose (0.1 mM, 0.2 μC/μmole). (C) *A. nidulans* R139 grown on 50 mM glucose + 50 mM sodium acetate (○) or 50 mM glucose + 50 mM sodium acetate + 50 mM fructose (●), incubated in the presence of D-[$^{14}C_6$]fructose (0.1 mM; 0.125 μC/μmole).

Fig. 2. Inhibition of sugar transport by 2,4-dinitrophenol. Uptake of 2-deoxy-D-[$^{14}C_6$]glucose by *A. nidulans* R46 (A), D-[$^{14}C_6$]galactose by *A. nidulans* R31 (B), and D-[$^{14}C_6$]fructose by *A. nidulans* R139 (C), in the absence (○) and presence (●) of 1 mM 2,4-dinitrophenol. In all cases the isotopic sugar concentration was 0.1 mM with a specific radioactivity of 0.2 μC/μmole.

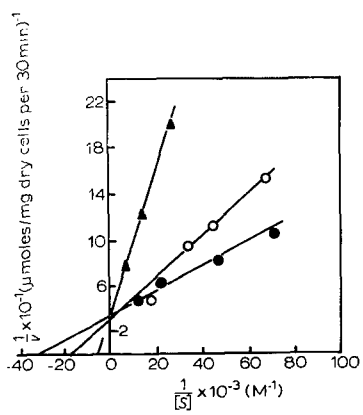
TABLE I

CONCENTRATION OF SUGARS FROM THE MEDIUM BY *A. nidulans*

Cells grown on 50 mM D-glucose + 50 mM sodium acetate were incubated in the presence of the isotopic sugar indicated. Uptake of isotopic was measured over a 30-min period; residual isotope in the medium was also determined. Intracellular concentration of sugar was calculated on the basis of 4 μl water per mg dry wt. cells. Uptake of 2-deoxy-D-[$^{14}C_6$]glucose (0.2 μC/μmole) was measured with *A. nidulans* R46; D-[$^{14}C_6$]galactose (0.2 μC/μmole) with *A. nidulans* R31, and D-[$^{14}C_6$]fructose (0.25 μC/μmole) with *A. nidulans* R139.

Sugar	*External concentration (mM)*		*Intracellular concentration (mM)*	*Concentration factor*
	Initial	*Final*		
2-Deoxy-D-[$^{14}C_6$]glucose	0.220	0.210	4.6	22
	0.100	0.044	1.8	41
	0.021	0.013	1.4	107
D-[$^{14}C_6$]Galactose	0.100	0.095	6.0	63
	0.053	0.047	4.6	98
	0.026	0.023	3.5	152
D-[$^{14}C_6$]Fructose	1.30	1.200	9.8	8
	0.32	0.300	5.4	18
	0.03	0.020	0.7	35

$2 \cdot 10^{-4}$ M; 2-deoxy-D-glucose, $6 \cdot 10^{-5}$ M. The last value is close to that reported for glucose ($4 \cdot 10^{-5}$ M) by BROWN AND ROMANO[8]. It is of interest that while these three sugars varied over a 10-fold range with respect to their carrier-complexing capacity, as indicated by their respective apparent K_m values, and they are transported by distinct systems as will be shown later in this paper, they nevertheless all exhibited the same v_{max} (Fig. 3).



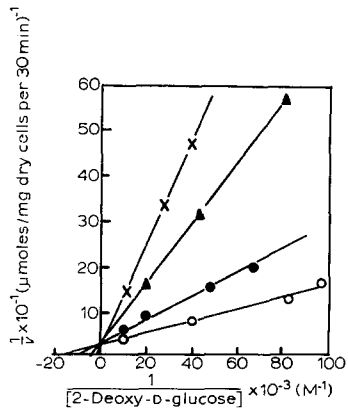


Fig. 3. Lineweaver–Burk plot of the uptake of 2-deoxy-D-[$^{14}C_6$]glucose by *A. nidulans* R46 (○), D-[$^{14}C_6$]galactose by *A. nidulans* R31 (●), and D-[$^{14}C_6$]fructose by *A. nidulans* R139 (▲). Specific activity of isotopic sugars was 0.2 μC/μmole.

Fig. 4. Lineweaver–Burk plot of the inhibition of 2-deoxy-D-glucose uptake by D-glucose, D-galactose, and D-mannose. *A. nidulans* R46 incubated with 2-deoxy-D-[$^{14}C_6$]glucose (0.2 μC/μmole) with no further addition (○), or with 1 mM D-glucose (×), 5 mM D-galactose (▲), or 1 mM D-mannose (●).

Specificity of the D-*glucose system*

Lineweaver–Burk plots of the inhibition of 2-deoxy-D-glucose uptake by D-glucose, D-galactose, and D-mannose are shown in Fig. 4. It is clear that criteria of competitive inhibition are satisfied. The apparent K_i for D-glucose calculated from these data is $6 \cdot 10^{-5}$ M, a value equal to the apparent K_m for 2-deoxy-D-glucose uptake. Thus, the latter non-metabolizable glucose analog definitely is transported by the glucose carrier. Other sugars and sugar derivatives can be ordered with respect to their glucose carrier–complexing capacity, therefore, by determining the degree to which they can competitively inhibit the uptake of 2-deoxy-D-glucose.

The results of such a study are shown in Table II, where the sugars tested are listed, together with the change in the structure of D-glucose which each represents, and the apparent K_i obtained. The following points can be made from these data:

TABLE II

INHIBITION OF 2-DEOXY-D-[$^{14}C_6$]GLUCOSE UPTAKE BY *A. nidulans* R46.

Cells grown on 50 mM D-glucose + 50 mM sodium acetate were incubated with 0.1 mM 2-deoxy-D-[$^{14}C_6$]glucose (0.2 μC/μmole). Non-isotopic sugars tested as competitors were added at concentrations of 1–10 mM and uptake of isotope was measured over a 30-min period. All sugars were considered as C1 chairs except L-glucose, L-xylose, L-sorbose, and D-fructose which were considered in the 1C conformation.

Sugar	*Changes of the C1 D-glucopyranose structure*	*Apparent K_i (mM)*
D-Glucose	—	0.06
1,5-Anhydroglucitol	1-Deoxy	1.5
α-Methyl-D-glucoside	α, 1-*O*-methyl	N.I.*
D-Mannose	2 axial OH	1.3
3-*O* Methyl-D-glucose	3-*O*-methyl	0.14
D-Galactose	4 axial OH	1.1
D-Xylose	5-dehydroxymethyl	3.8
6-Deoxy-D-glucose	6-deoxy	0.38
D-Fucose	6-deoxy, 4 axial OH	N.I.
D-Rhamnose	6-deoxy, 3 axial OH	N.I.
L-Arabinose	5-dehydroxymethyl, 4 axial OH	N.I.
L-Glucose	1-CH_2OH, 5-OH (anomeric)	N.I.
L-Xylose	1-deoxy, 5-OH (anomeric)	N.I.
L-Sorbose	1-deoxy, 5-(CH_2OH + OH) (anomeric)	N.I.
D-Fructose	1-deoxy, 2 axial OH 5-(CH_2OH + OH) (anomeric)	N.I.

* N.I. = no inhibition. $K_i > 50$ mM.

Only those sugars which differ from the C1 D-glucopyranose structure by a single change compete for uptake. However, alteration of a substituent group at any one of the six carbon positions can be tolerated. Except for methylation of the anomeric hydroxyl group, (α-methyl-D-glucoside) no single change completely abolished binding activity. In all cases, however, the combination of more than one change resulted in complete loss of carrier affinity. Thus, a combination of an axial 4-OH (D-galactose) and the absence of the 5-CH_2OH (D-xylose), each of which when occurring alone, only decreased activity, resulted in complete loss of carrier affinity (L-arabinose). The low activities of L-xylose, L-sorbose, and D-fructose also represent loss of activity as a result of multiple changes. These sugars, as well as L-glucose, would be

expected to occur in solution as 1C chairs. They would, therefore, represent analogues of the C1 D-glucopyranose structure in which the substituents of carbons 1 and 5 and carbons 2 and 4 are reversed. The low activity of L-glucose could be due to the presence of both the hydroxymethyl group at the position corresponding to carbon 1 of the C1 D-glucopyranose and the hydroxyl group of the corresponding C-5 position, or result from the presence of either one of these groups alone. The equally low activity of α-methyl-D-glucoside suggests that the 1-CH_2OH alone can account for the low carrier affinity of L-glucose. The 5-OH could well reduce affinity further.

The substrate specificity of the D-glucose transport system does not seem to be dependent on any single ring substituent. The moderate activity of 1,5-anhydroglucitol shows that, while the anomeric hydroxyl group contributes to sugar activity, it is not essential. Although the anomeric hydroxyl group may be removed, glycosidic linkage to a methyl group (α-methyl-D-glucoside) or another sugar group (sucrose) abolished activity. This suggests that a group other than H or OH at C-1 causes steric interference during sugar carrier association. The equatorial hydroxyl group at C-2 does not contribute to activity since the K_m's for D-glucose and 2-deoxy-D-glucose uptake are the same. However, addition of an axial hydroxyl group at C-2 (D-mannose) causes significant loss of activity, presumably due to steric interference during carrier binding. The relatively high activities of 3-*O*-methyl-D-glucose and 6-deoxy-D-glucose show that neither the 3-OH group nor the 6-OH group are essential for sugar activity, although both contribute. The further decrease in activity resulting from loss of both the 6-OH group and C-6 (D-xylose) indicates that C-6 itself contributes to substrate specificity. The decreased binding activity of D-galactose shows that the equatorial 4-OH group is more active than the axial 4-OH group, but whether this decreased activity results from steric interference by the axial 4-OH group or from loss of the equatorial 4-OH group cannot be determined.

Specificity of the D-*galactose transport system*

Table III lists the apparent K_i values obtained from studies of the inhibition of D-galactose uptake using the galactokinaseless strain R31. Comparison of the apparent

TABLE III

INHIBITION OF D-[$^{14}C_6$]GALACTOSE UPTAKE IN *A. nidulans* R31 (GALACTOKINASELESS) (D-GALACTOSE K_m = 0.03 mM)

Cells were grown on 50 mM glucose + 50 mM sodium acetate and incubated in the presence of 0.1 mM D-[$^{14}C_6$]galactose (0.2 μC/μmole). Non-isotopic sugars were added at concentrations of 1–10 mM and uptake of isotope was measured over a 30-min period. All sugars were considered as C1 chairs.

Sugar	*Changes of the C1 D-galactopyranose structure*	*Apparent K_i (mM)*
D-Fucose	6-deoxy	0.29
L-Arabinose	5-dehydroxymethyl	10.50
D-Glucose	4 equatorial OH	0.50
2-Deoxy-D-glucose	4 equatorial OH, 2-deoxy	0.36
D-Mannose	4 equatorial OH, 2 axial OH	0.59
6-Deoxy-D-glucose	4 equatorial OH, 6-deoxy	N.I.*
D-Xylose	4 equatorial OH, 5-dehydroxymethyl	N.I.
D-Rhamnose	4 equatorial OH, 2 axial OH, 6-deoxy	N.I.
α-Methyl-D-galactoside	1-*O*-methyl	N.I.

* N.I. = no inhibition. $K_i > 50$ mM.

K_i values with the K_m for D-galactose transport indicates that each competing sugar has a reduced carrier affinity by comparison to D-galactose. No inhibition was exhibited by 6-deoxy-D-glucose, D-xylose, D-fructose, L-xylose, L-sorbose, D-arabinose, D-rhamnose, lactose and α- and β-methyl-D-galactopyranoside.

As in the case of the D-glucose transport system, no single substituent on the galactopyranose ring appears absolutely necessary for substrate activity for the D-galactose transport system in *A. nidulans*. The following preliminary conclusions can be stated about the specific structural contributions of each ring substituent. An axial 4-OH group is more active than equatorial 4-OH group since D-glucose, which differs from D-galactose only by inversion of the 4-OH group to the equatorial position, showed a decreased affinity for the carrier. Inhibition by both D-fucose and L-arabinose shows that, while both the 5-CH_2OH group and the hydroxyl group on this carbon (6-OH) contribute to sugar activity, neither is essential. It is significant that, while loss of the 6-OH group (D-fucose) resulted in only a moderate decrease in activity loss of this OH group *plus* C-6 (L-arabinose) further decreased activity by two orders of magnitude. The equatorial 2-OH group does not appear to contribute to transport specificity since the K_i values of 2-deoxyglucose, which lacks this hydroxyl group, and of D-glucose are very similar. Axial addition of an hydroxyl group at C-2 does not seem to cause steric interference for carrier association since the K_i value of D-mannose, which has the 2-OH group in the axial position, is practically identical with that of D-glucose. As in the case of glucose transport, methylation of the anomeric hydroxyl group and all multiple changes not involving substituents on C-2 resulted in complete loss of carrier-binding activity.

Although D-galactose has been shown to exhibit binding activity with the D-glucose transport carrier and can presumably be transported by the D-glucose system, D-galactose and D-glucose transport can be clearly differentiated on the basis of substrate specificity and affinity. This is shown in Table IV. 6-Deoxyglucose and D-xylose competed for uptake with 2-deoxyglucose but not with galactose. Therefore,

TABLE IV

COMPARATIVE INHIBITION OF 2-DEOXY-D-[$^{14}C_6$]GLUCOSE AND D-[$^{14}C_6$]GALACTOSE UPTAKE BY VARIOUS SUGARS (EXPRESSED AS K_i)

A. nidulans R46 and R31 were grown on 50 mM D-glucose + 50 mM sodium acetate and incubated in the presence of 0.1 mM (0.2 μC/μmole) 2-deoxy-D-[$^{14}C_6$]glucose or D-[$^{14}C_6$]galactose, respectively. Non-isotopic sugars tested as competitors were added at concentration of 10 mM, and uptake of isotope was measured over a 30-min period.

Sugar	*K_i value (M) Inhibition of uptake of:*	
	2-Deoxy-D-glucose	*D-Galactose*
D-Glucose	$6.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-4}$
D-Mannose	$1.3 \cdot 10^{-3}$	$5.9 \cdot 10^{-4}$
D-Fucose	N.I. *	$2.9 \cdot 10^{-4}$
L-Arabinose	N.I.	$1.1 \cdot 10^{-2}$
D-Xylose	$3.8 \cdot 10^{-3}$	N.I.
6-Deoxy-D-glucose	$3.8 \cdot 10^{-4}$	N.I.

* N.I. = no inhibition. $K_i > 50$ mM.

these sugars must be transported by the D-glucose system but not by the D-galactose system. Conversely, D-fucose and L-arabinose competed for uptake with galactose but not with 2-deoxy-D-glucose and are, therefore, transported only by the D-galactose system. D-Glucose and D-mannose appear to be transported by both systems, but the affinities of these sugars for each system differ by one order of magnitude. Two separate systems are clearly indicated, therefore.

Specificity of D-fructose transport

The uptake of D-fructose appears to be mediated by a highly specific carrier, since it was completely unaffected by the addition of a number of analogues and possible competitors, even when these were added at concentrations 100 times greater than that of D-fructose. Sugars tested included D-glucose, D-galactose, D-mannose, L-sorbose, L-arabinose, D-arabinose, D-xylose, L-xylose, sucrose, sorbitol, and mannitol.

Comparison of A. nidulans with other organisms

Comparison of the fungal system described here with those of yeast and mammalian red blood cells, as described by CIRILLO[7] and LEFEVRE[11], respectively, reveals a pattern of broad similarity with respect to specificity of the glucose transport system. Comparative data are shown in Table V. There are differences, and *A. nidulans* appears to show stricter specificity than either yeast or erythrocytes. The *A. nidulans* glucose carrier has essentially no affinity for D-fucose, D-arabinose, and like the erythrocyte system, has no affinity for L-sorbose or D-fructose as does the yeast system. The apparent affinity of D-fructose in yeast is anomalous, however[7]. Nevertheless, the overall pattern is one of broad similarity; this may indicate fundamental similarities in the carrier systems of eukaryotic cells.

The specificity pattern is in strong contrast, however, to that found in bacteria, at least as represented by *Escherichia coli*. First of all, α-methyl glucoside, which is

TABLE V

COMPARATIVE AFFINITIES* OF SUGARS AS SUBSTRATES FOR GLUCOSE TRANSPORT SYSTEMS IN *A. nidulans*, YEAST, AND ERYTHROCYTES

Sugar	*A. nidulans*	*Yeast***	*Erythrocyte****
D-Glucose	1	1	1
2-Deoxy-D-glucose	1	1	1.4
D-Fructose	<0.0012	0.2	0.0025
D-Galactose	0.055	0.13	0.2
D-Mannose	0.046	0.1	0.3
1,5-Anhydro-D-glucitol	0.04	0.1	0.2
3-*O*-Methyl-D-glucose	0.48	0.02	—
D-Xylose	0.016	0.13	0.11
D-Fucose	<0.0012	0.02	0.03
D-Arabinose	<0.0012	0.02	0.005
L-Sorbose	<0.0012	0.005	<0.0025
L-Glucose	<0.0012	0.0025	<0.0025
α-Methyl-D-glucoside	<0.0012	0.0025	<0.0025

* Expressed as a ratio of K_m or K_i of D-glucose to that of the sugar listed.
** From CIRILLO[7].
*** From LEFEVRE[11], and cited by CIRILLO[7].

inert in the fungus system, is actively transported by bacteria[18], and is the non-metabolizable analog of glucose most commonly used in transport studies. Secondly, while 6-deoxy-D-glucose competitively inhibits 2-deoxy-D-glucose uptake in *A. nidulans*, and is actively transported[8], this analog is inert in *E. coli*; thus, ROGERS AND YU[12] identified the free hydroxyl at carbon 6 of glucose as the single most important functional group. This is explainable on the basis that glucose is transported by *E. coli via* the phosphoenolpyruvate phosphotransferase system[5,19]. In this system, glucose and its analogues are phosphorylated at the C-6 hydroxyl group during transport; thus, a free C-6 hydroxyl group is essential for transport. In contrast, this system is not operative in *A. nidulans*. Glucose transport takes place independently of phosphorylation, as shown by BROWN AND ROMANO[8]; and there is no requirement for a free C-6 hydroxyl group. It is of interest that in certain strictly aerobic bacteria, such as *Pseudomonas aeruginosa* and *Arthrobacter globiformis* which lack the phophoenolpyruvate phosphotransferase system, 6-deoxy-D-glucose competitively inhibits 2-deoxy-D-glucose uptake[20].

Acetate inhibits the uptake of a number of sugars by *A. nidulans*, including a number of disaccharides and sugar alcohols in addition to the hexoses that were studied in the present work[2]. And since it is clear that D-glucose, D-fructose, and D-galactose are each transported by distinct systems, and similar specificities must exist for the other sugars, one must conclude that either each separate permease has a common biochemical feature that renders it sensitive to the acetate effect, or that sugar transport involves an additional common element that is distinct from the stereospecific carrier. Though data are insufficient to allow a choice between these possibilities, the latter seems more likely. The fact that D-glucose, D-galactose and D-fructose use separate carriers and show different K_m values for transport, but exhibit the same v_{max} may indicate the participation of a common rate-limiting element.

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