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SUGAR TRANSPORT IN *COPRINUS CINEREUS*

DAVID MOORE and MAGDALENE S. DEVADATHAM
Department of Botany, The University, Manchester M13 9PL (U.K.)
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Summary

Two transport systems for glucose were detected: a high affinity system with a K_m of 27 μM, and a low affinity system with a K_m of 3.3 mM. The high affinity system transported glucose, 2-deoxy-D-glucose (K_m = 26 μM), 3-*O*-methylglucose (K_m = 19 μM), D-glucosamine (K_m = 652 μM), D-fructose (K_m = 2.3 mM) and L-sorbose (K_m = 2.2 mM). All sugars were accumulated against concentration gradients. The high affinity system was strongly or completely inhibited by *N*-ethylmaleimide, quercetin, 2,4-dinitrophenol and sodium azide. The system had a distinct pH optimum (7.4) and optimum temperature (45°C). The low affinity system transported glucose, 2-deoxy-D-glucose (K_m = 7.5 mM), and 3-*O*-methylglucose (K_m = 1.5 mM). Accumulation again occurred against a concentration gradient. The low affinity system was inhibited by *N*-ethylmaleimide, quercetin and 2,4-dinitrophenol, but not by sodium azide. The rate of uptake by the low affinity system was constant over a wide temperature range (30—50°C) and was not much affected by pH; but as the pH of the medium was altered from 4.5 to 8.9 a co-ordinated increase in affinity for 2-deoxy-D-glucose (from 52.1 mM to 0.3 mM) and decrease in maximum velocity (by a factor of five) occurred. Both uptake systems were present in sporelings germinated in media containing sodium acetate as sole carbon source. Only the low affinity system could initially be demonstrated in glucose-grown tissue, although the high affinity system was restored by starvation in glucose-free medium. The half-time for restoration of high affinity activity was 3.5 min and the process was unaffected by cycloheximide. Addition of glucose to an acetate-grown culture inactivated the high affinity system with a half-life of 5—7.5 s. Addition of cycloheximide to an acetate-grown culture caused decay of the high affinity system with a half-life of 80 min. Regulation is thus thought to depend on modulation of protein activity rather than synthesis, and the kinetics of glucose, 2-deoxy-D-glucose and 3-*O*-methylglucose uptake would be consistent with there being a single carrier showing negative co-operativity.

Abbreviation: NCM, 30 mM NH_4Cl, 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 2 mM Na_2SO_4, 0.5 mM $MgSO_4$ and 1.5 μM thiamin hydrochloride.

Analysis of transport defective mutants revealed defects in both transport systems although the mutants used were alleles of a single gene. It is concluded that this gene (the *ftr* cistron) is the structural gene for an allosteric molecule which serves both transport systems.

Introduction

Interest in the mechanism of sugar uptake in *Coprinus* arose from work done with transport-defective mutants called *ftr* mutants. Though originally selected as being resistant to growth inhibitions caused by 2-deoxy-D-glucose [1], they have also been obtained by selecting for resistance to inhibition by L-sorbose or D-glucosamine [2]. An unselected feature of their phenotype is that *ftr* mutants grow very poorly with fructose as sole carbon source and since the prototroph grows well on fructose this feature provides a reverse selective procedure (i.e., from mutant to wild type) which can be employed for study of allelic recombination [3,4], of the mutagen sensitivity of different parts of the gene [5] and of the effect of different levels of selection pressure on different parts of the gene [6]. These genetic studies unequivocally show that all *ftr* mutants isolated to date (whatever the selection procedure used) are alleles of a single gene. Physiological studies strongly suggest that the product of this gene is involved in sugar transport. To provide an adequate biochemical background for these genetic studies the mechanism of hexose uptake in the prototroph is here examined in some detail.

Materials and Methods

Organism and culture conditions. Germinated oidia of homokaryon BC9/6,6 of the basidiomycete *Coprinus cinereus* (Schaeff. ex Fr.) S.F. Gray were used throughout. This is the same prototroph used (under the name *Coprinus lagopus*) for the isolation of *ftr* mutants. Oidia were routinely germinated in a defined liquid medium containing 25 mM sodium acetate as sole carbon source. The basal medium used, called NCM [7], was also employed in the experiments on sugar accumulation described below; it contained 30 mM NH_4Cl, 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 2 mM Na_2SO_4, 0.5 mM $MgSO_4$ and 1.5 μM thiamin hydrochloride and had a pH of 6.8. Spore suspensions were incubated at 37° C on an angled turntable for 16—18 h. Gentle centrifugation was then used to sediment the young mycelia sufficiently to allow removal of the medium and ungerminated (and unsedimented) spores [8]. A series of such washes brought the sporelings into suspension in fresh (carbon-free) NCM medium to a concentration corresponding to 1—2 mg dry wt./ml. For some experiments this routine method was varied, e.g., by germination in NCM + 25 mM glucose, or by resuspension of sporelings in experimental media of different pH or ionic composition. Unless specifically noted to the contrary, however, all tissue used in the experiments described below was germinated in medium containing acetate as sole carbon source. Non-radioactive chemicals were obtained from B.D.H., Hopkin and Williams, Koch-Light Laboratories and Sigma Chemical Co.; they

were of AnalaR grade or better. Sugars used in uptake experiments were chromatographically homogeneous.

Uptake measurements. The physiological phenotype of *ftr* mutants formed the basis for the design of work to be done with the prototroph and required study of the uptake of glucose, fructose, 2-deoxy-D-glucose, sorbose and glucosamine; 3-*O*-methylglucose was included as it has been the most widely used sugar analogue in comparable studies of sugar uptake in other filamentous fungi. Sugar accumulation was studied by exposing sporeling suspensions to isotopically labelled sugar solutions. The radioactive sugars, D-[U-^{14}C]glucose, D-[U-^{14}C]fructose, 2-deoxy-D-[1-^{3}H]glucose, 3-*O*-methyl-D-[U-^{14}C]glucose, D-[1-^{14}C]glucosamine hydrochloride, and L-[U-^{14}C]sorbose, were obtained from the Radiochemical Centre, Amersham. Accumulation experiments were done at 37° C in a final volume of 25 ml of NCM medium containing the tissue and appropriate sugar. Reactions were initiated by addition of the tissue and at intervals of 60—80 s samples of 2 or 4 ml were removed, filtered through Whatman GF/A filters and washed twice with 50 ml of ice-cold water. The filters with their adhering sample of tissue were placed directly into vials of scintillation fluid. Sugar concentrations in the experimental vessel ranged from 1 μM to 25 mM as required by the experiment; tissue concentration was adjusted so that each sample consisted of 80—160 μg dry wt. Radioactivity was varied but was usually in the range 0.1—1.0 μCi · ml^{-1} and the specific activity of each reaction vessel was determined by measuring the radioactivity of three separate 1 μl samples of the medium (removed before addition of tissue). The scintillation fluid consisted of a 2 : 1 mixture of toluene and Triton X-100, containing 5 g · l^{-1} of diphenol oxazole. Counting was done in a Packard Model 2420 LS spectrometer.

Interpretation. The standard assays were done as described with NCM basal medium (pH 6.8) and a temperature of 37° C; five samples of 4 ml being removed 30, 110, 190, 270 and 350 s after first challenge with the radioactive medium. Accumulation of all six sugars was found to be proportional to time of exposure within this time interval (Fig. 1). However, as noted for *Neurospora* [9] uptake vs. time plots did not always extrapolate to zero because of non-specific adsorption. As with *Neurospora*, the amount of sugar adsorbed could be determined from appropriate control experiments using tissue killed either by heat or by exposure to formaldehyde. In the majority of experiments, though, only the rate of uptake was of interest and as the non-specific adherence of sugar simply altered the baseline of the uptake vs. time curve the rate was calculated as the regression slope for the data obtained without attempt to correct for non-zero intercept on the v axis. Initial rates calculated for the interval between 30 and 350 s were used to assess inhibitions and determine kinetic constants. As the work progressed it became clear that two different kinetic entities contributed to the uptake of certain sugars (Fig. 2). The two systems differed so drastically in substrate affinity (Table V) that the potential contribution of the low affinity uptake system to accumulation at substrate concentrations appropriate to the high affinity one was proportionately very much less than the normally experienced standard errors and was thus deemed to be negligible. Similarly, contribution of the high affinity system to uptake at substrate concentrations appropriate to kinetic analysis of

the low affinity uptake process was considered negligible because the evidence indicated that such substrate concentrations inactivated the high affinity mechanism. Consequently, kinetic parameters for each system were separately determined using $[S]/v \times [S]$ plots of data from experiments which employed substrate concentrations appropriate to the system under investigation and without reference to the alternative uptake system. Experiments with sugar mixtures revealed strictly competitive interactions as judged from plots of $1/v \times 1/[S]$, $1/v \times [I]$, and $[S]/v \times [S]$. Substrate affinities were established using the approach detailed by Cirillo [10]. For the 23 pair-wise sugar combinations used the effect of 2—4 concentrations of (unlabelled) inhibitor on the rate of uptake of each of 2—4 concentrations of (isotopically labelled) substrate was measured, giving 4—9 independent estimates of apparent K_i.

The specific activity of the reaction mixture, the radioactivity of the tissue sample and the dry weight of the tissue samples were all measured directly, allowing sugar accumulation to be immediately expressed in terms of moles of sugar accumulated in unit time by unit dry weight of tissue. These values were converted to intracellular concentrations when necessary by making allowance for the intracellular volume which was determined as the volume of water in packed tissue samples which was unavailable to inulin-[^{14}C]carboxylic acid [11]. The same value (2.1 μl (mg dry wt.)$^{-1}$) was obtained with both germinated and ungerminated oidia.

Results and Discussion

Evidence for two transport systems

All of the sugars were accumulated to intracellular concentrations which greatly exceeded the external sugar concentration (Fig. 1). Results obtained with glucose, fructose and glucosamine were concordant with those obtained with the unnatural sugar analogues 2-deoxy-D-glucose, 3-*O*-methylglucose and sorbose, so it is assumed that this accumulation truly represents uphill transport rather than apparent accumulation due to metabolism subsequent to transport. Analysis in Lineweaver-Burk plots separated the sugars into two groups (Fig. 2). Fructose, glucosamine and sorbose formed single lines, whereas glucose, 2-deoxy-D-glucose and 3-*O*-methylglucose data revealed two different kinetic entities by forming biphasic plots. Assessment of inhibition relationships between the sugars suggests that it is necessary to postulate only two uptake systems to satisfy these data. Glucose and 2-deoxy-D-glucose in the concentration range 1—50 μM both competitively inhibited uptake of fructose and sorbose, K_m and K_i values being very similar (Table I). This is taken to mean that a single carrier is responsible for uptake of fructose, sorbose and (in the 1—50 μM concentration range) glucose and 2-deoxy-D-glucose; since 3-*O*-methylglucose at low concentration was also a competitive inhibitor of sorbose uptake we assume that this sugar is also transported by this carrier when present at low concentration. We describe this as the high affinity transport system since the apparent K_m for glucose was 27 μM.

In the high concentration range (1—20 mM) a much lower affinity for glucose, 2-deoxy-D-glucose and 3-*O*-methylglucose was revealed, yet within this range of concentrations these three sugars again showed competitive inhibi-

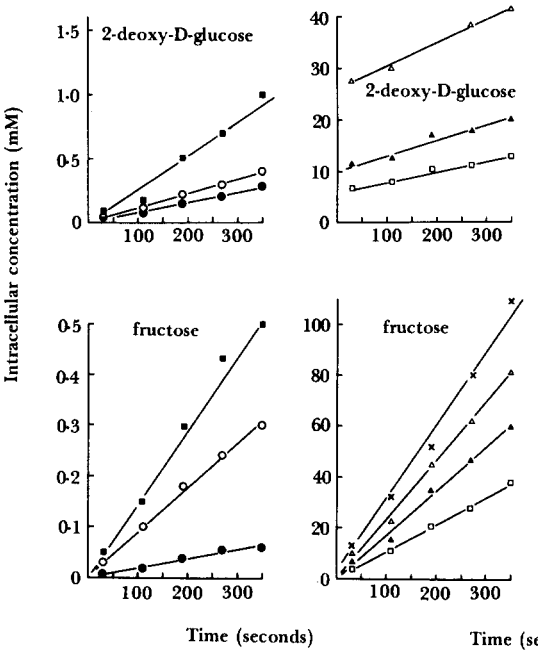

Fig. 1. Accumulation of sugars by young mycelia of *Coprinus cinereus*. Experiments were performed with sporelings germinated in acetate medium. The uptake assay used NCM medium (pH 6.8) as the basal solution and was done at 37°C. Initial sugar concentration in the medium is indicated by the symbols: •, 0.001 mM; ○, 0.005 mM; ■, 0.01 mM; □, 1.0 mM; ▲, 5 mM; △, 10 mM; X, 20 mM. Failure to extrapolate to the origin is due to non-specific adsorption of sugar to the mycelium; it is sugar and concentration dependent and though compensating corrections can be applied (see text) none were applied to these data. Net accumulation due to transport alone is readily estimated as the difference between the internal concentrations of the 30- and 350-s samples of tissue. In every case net accumulation exceeds the initial sugar concentration in the medium, showing that uptake occurs against a concentration gradient.

tions with similar K_m and K_i values. Thus we conclude that a single carrier is able to transport all three sugars in this concentration range and describe it as the low affinity transport system as the apparent K_m for glucose was 3.3 mM.

Glucosamine and fructose were able to act as competitive inhibitors of 2-deoxy-D-glucose uptake in the high concentration range, but the K_i values obtained (Table II) were so different from the K_m values got from experiments where glucosamine or fructose were used alone that we conclude that the K_i and K_m measures here refer to different kinetic systems. We assign the sugar substrates to the two uptake systems in the pattern indicated in Tables I and II. Fructose, sorbose and glucosamine were transported only by the high affinity system; glucose, 2-deoxy-D-glucose and 3-*O*-methylglucose were transported either by the high or the low affinity system depending on their concentration in the suspending medium. For experimental purposes the two systems were readily distinguished either by sugar identity (e.g., sorbose was transported only by the high affinity system) or by sugar concentration (e.g., 1—50 μM 2-deoxy-D-glucose was transported by the high affinity system, but 1—20 mM 2-deoxy-D-glucose was transported by the low afffinity system, see Fig. 2). Thus the characteristics of the two systems could be separately established quite easily.

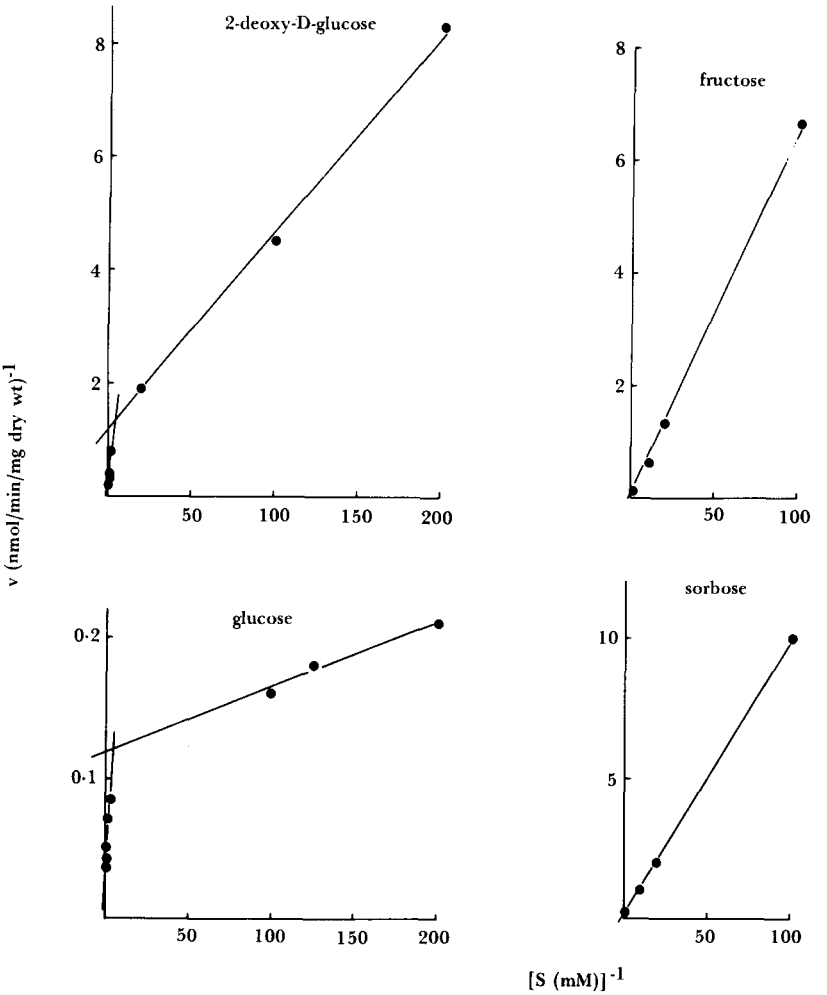

Fig. 2. Double-reciprocal plots showing the kinetics of sugar uptake by young mycelia of *Coprinus cinereus*. These data derive from single representative experiments chosen and drawn here specifically to illustrate the point that some sugars show biphasic plots while the others do not. In replicate experiments involving independent tissue preparations values for K_m were reproducible (±15%) but rates of uptake (and hence estimates of V) differed drastically. The rate of uptake is expressed in terms of tissue dry weight. Germinating sporelings become progressively more vacuolated as they develop and grow, and as this inevitably means that the quantity of cell wall increases in varying proportion to increase in cytoplasm the variation in uptake rate is presumed to result from variation in the relation between the dry weight of the tissue and the activity of its cytoplasm. Consequently, estimates of V can only be reliably compared when experiments are done with the same tissue preparation (e.g., Table V). This restriction on comparability does not apply to estimates of K_m or apparent K_i.

It is necessary to stress that no evidence has been obtained which requires any further hexose transport system to be postulated. This is particularly important for fructose and sorbose uptake. In *Aspergillus nidulans* fructose was transported by a highly specific carrier insensitive to glucose inhibition [12] and an independent fructose transport system was also identified in ungerminated conidia of *Neurospora* [11]. All of the evidence obtained with germinated spores of *Coprinus* is consistent with the view that both sorbose

TABLE I

KINETIC CONSTANTS OF THE HIGH AFFINITY GLUCOSE TRANSPORT SYSTEM OF *COPRINUS CINEREUS*

Experiments were done with sporelings germinated for 16—18 h in medium containing sodium acetate as sole carbon source. Uptake was measured at 37°C using NCM medium (pH 6.8) as the basal solution. Values of K_m (mM) and V (nmol · min^{-1} (mg dry wt.)$^{-1}$) were determined graphically and are shown here with their appropriate standard errors. Values of apparent K_i (mM) were calculated using the method of Cirillo [10] and the means of 4—9 estimates are recorded here. Coefficients of variation ranged from 12% to 85% and averaged 44%. Relative affinities are expressed as the ratio of the K_m value for glucose to that of the listed sugar, or, where only K_i estimates are available for comparison, as the ratio of the apparent K_i for inhibition of sorbose uptake by glucose to the apparent K_i for inhibition of sorbose uptake by the listed sugar. Values of V are estimated in terms of unit dry wt. of tissue and were found to differ quite widely between different samples of tissue in a way which seemed to depend on the degree of vacuolation of the hyphae. This limits the usefulness of these rate estimates for comparative purposes but did not seriously affect K_m or K_i estimates.

Substrate	K_m	V	Approx. K_i for inhibition of sorbose uptake *	Approx. K_i for inhibition of fructose uptake *	Approx. K_i for inhibition of 2-deoxy-D-glucose uptake *	Relative affinity
D-Glucose	0.027 ± 0.003	7.3 ± 0.8	0.013	—	—	1
2-Deoxy-D-glucose	0.026 ± 0.006	0.8 ± 0.2	0.024	0.08	—	1.04
3-*O*-Methylglucose	0.019 ± 0.001	1.3 ± 0.1	0.037	—	—	1.42
D-Glucosamine	0.652 ± 0.155	15.9 ± 3.8	—	—	—	0.04
D-Fructose	2.28 ± 0.03	35.5 ± 0.5	1.41	—	3.4	0.01
L-Sorbose	2.24 ± 0.07	25.0 ± 0.8	—	2.23	1.7	0.01
Methyl-α-glucoside	—	—	0.38	—	—	0.03
Methyl-β-glucoside	—	—	2.18	—	—	0.006
D-Galactose	—	—	0.30	—	—	0.04
D-Xylose	—	—	0.14	—	—	0.09
6-Deoxy-D-glucose	—	—	0.02	—	—	0.65
Glucuronic acid	—	—	0.13	—	—	0.1

* When employed as substrates for inhibition experiments sorbose and fructose were used in the concentration range 2—6 mM and 2-deoxy-D-glucose in the range 0.05—0.5 mM.

and fructose were transported only by the glucose high affinity carrier, a similar situation to that which obtains in mycelia and germinated conidia of *Neurospora* [13].

Characteristics of the two transport systems

Both systems accumulated against concentration gradients (Fig. 1) and both were sensitive to inhibition by metabolic poisons. The rates of uptake of 3 mM sorbose or 15 mM 2-deoxy-D-glucose were inhibited 30—50% by inclusion of 5 mM malonate or 5 mM iodoacetate in the reaction vessel. Uptake from 3 mM solutions of sorbose was almost totally prevented (86—96% inhibition) by inclusion of either 5 mM sodium azide or 5 mM 2,4-dinitrophenol. On the other hand, uptake from 10 mM solutions of 2-deoxy-D-glucose, though almost completely inhibited (99%) by 2,4-dinitrophenol, was essentially insensitive to sodium azide (14% inhibition). Differential sensitivity to azide was confirmed, and sensitivity to some other inhibitors compared, by experiment with widely different initial concentrations of 2-deoxy-D-glucose

TABLE II

KINETIC CONSTANTS OF THE LOW AFFINITY GLUCOSE TRANSPORT SYSTEM OF *COPRINUS CINEREUS*

Experiments used sporelings germinated for 16—18 h in medium containing sodium acetate as sole carbon source and were carried out and interpreted as described in the legend to Table I. Coefficients of variation of the estimates of apparent K_i ranged from 17% to 65% and averaged 40%. nt, not transported.

Substrate	K_m	V	Approx. K_i for inhibition of 2-deoxy-D-glucose uptake *	Relative affinity
D-Glucose	3.3 ± 0.04	48.2 ± 0.6	4.0	1
2-Deoxy-D-glucose	7.5 ± 0.82	7.1 ± 0.8	—	0.44
3-*O*-Methylglucose	1.5 ± 0.05	3.1 ± 0.1	2.5	2.20
D-Glucosamine	nt	nt	102.4	0.04
D-Fructose	nt	nt	7.9	0.51
Methyl-α-glucoside	—	—	129.3	0.03
Methyl-β-glucoside	—	—	50.9	0.08
D-Galactose	—	—	156.3	0.03
D-Xylose	—	—	25.3	0.16
Glucuronic acid	—	—	7.6	0.53

* When employed as substrate for these inhibition experiments 2-deoxy-D-glucose was used in the concentration range 10—20 mM.

(Table III). Both systems clearly depended on supply of metabolic energy and operation of an ATPase. The differential sensitivity to azide is very puzzling, but it provides a clear distinction between the two systems and may reflect a difference in the way they are energised.

The two systems differed in response to change of temperature or pH of the suspending medium (Table IV). The high affinity system had a clear optimum of both temperature and pH for sorbose uptake while the low affinity system maintained a fairly constant uptake rate over wide pH and temperature ranges.

TABLE III

EFFECTS OF SOME INHIBITORS ON THE RATE OF UPTAKE OF 2-DEOXY-D-GLUCOSE

Inhibitor	Percent inhibition of initial uptake rate from medium containing:	
	10 μM 2-deoxy-D-glucose (high affinity uptake system)	2 mM 2-deoxy-D-glucose (low affinity uptake system)
Sodium azide (5 mM)	100	0
2-*n*-Heptyl-4-hydroxy-quinoline-*N*-oxide (1 mM)	0	38
Rotenone (1 mM)	30	36
2,4-Dinitrophenol (5 mM)	70	85
Gramicidin (0.5 mM)	0	29
Quercetin (1 mM)	97	100
N-Ethylmaleimide (1 mM)	99	98

TABLE IV

EFFECTS OF TEMPERATURE AND pH ON UPTAKE OF SORBOSE AND 2-DEOXY-D-GLUCOSE

Entries show uptake rates as percentages of those measured under standard assay conditions (pH 6.8 and 37°C temperature) from solutions initially containing 3 mM L-sorbose to represent the high affinity uptake system, or 15 mM 2-deoxy-D-glucose to represent the low affinity system.

pH	Sorbose uptake	2-Deoxy-D-glucose uptake
5.9	39	102
6.8	100	100
7.4	135	100
8.0	100	84
Temperature (°C)		
27	89	73
37	100	100
42	171	103
47	171	103

The most dramatic effect of pH was on the kinetic constants of 2-deoxy-D-glucose uptake; those of the high affinity system remained virtually unchanged, but the K_m and maximum velocity of the low affinity system were both greatly reduced as the medium was made more alkaline (Table V). These changes in kinetic constants are such as to ensure a virtually uniform unidirectional flux from any particular sugar concentration over the entire pH range, so the phenomenon may have adaptive significance. Growth of *C. cinereus* in complex media has been shown to be accompanied by alkalinisation of the medium even if it was initially buffered [14—17]; generally, the most alkaline pH values were reached as the medium became exhausted of carbohydrate. The observed changes in kinetic constants of the low affinity carrier would assure both a constant rate of uptake as the pH altered and an increase in carrier affinity at those pH values which seem normally to be associated with greatly diminished sugar concentrations. An alternative interpretation might be that the decrease in affinity seen as the medium was made more acidic is a reflection of events which normally occur at the inner membrane face; i.e., that protonation of the

TABLE V

CHANGES IN KINETIC CONSTANTS OF 2-DEOXY-D-GLUCOSE UPTAKE WITH ADJUSTMENT OF pH OF THE MEDIUM

Entries show the values of K_m (mM) and V (nmol/mg dry wt. per min) at different initial pH levels. The two uptake systems were distinguished by using the substrate concentration ranges appropriate to each. The same germinated spore suspension was used throughout to ensure comparability of velocity measurements.

pH	High affinity system		Low affinity system	
	K_m	V	K_m	V
4.5	0.01	3.3	52.1	67.6
7.0	0.04	3.7	10.0	50.0
8.9	0.08	5.2	0.3	13.5

loaded carrier is employed to promote discharge of the transported substrate. This would be similar to, but the inverse of, hexose transport in *Chlorella* [18]. It is not impossible that both interpretations are applicable.

Substrate affinities of the two transport systems

By assessing the relative abilities of sugar analogues to inhibit accumulation of 0.3—12.0 mM sorbose (to characterise the high affinity system) or 10—20 mM 2-deoxy-D-glucose (to characterise the low affinity system) the relative affinities of the carriers were compared in the way described by Cirillo [10]. The apparent K_i values obtained and relative affinities calculated are listed in Tables I and II. The two systems were similar. For both, single changes at C-2, C-3 or C-6 were least disturbing but a change at any other position drastically affected interaction with the carrier. A feature of the relationship with the methylglucosides was that the low affinity carrier was much more sensitive to methylation in the α-anomeric position than to β-methylation; a response exactly opposite to that of the high affinity carrier.

Regulation of sugar transport

The high affinity transport system was observed in sugar-starved (i.e., acetate-grown) cells providing they were not exposed to glucose (or 2-deoxy-D-glucose or 3-*O*-methylglucose) concentrations greater than 500 μM. The low affinity system was observed, immediately and without any lag period, when such cells were exposed to glucose (or 2-deoxy-D-glucose or 3-*O*-methylglucose) concentrations greater than 500 μM; and it was the only transport system that could be demonstrated initially in cells germinated in medium containing glucose as sole carbon source. The high affinity system could be demonstrated in glucose-grown cells only after a period of starvation in sugar-free medium.

The regulatory effect of glucose suggested first a situation like that reported for *Neurospora* where one system is constitutive and the other is derepressed by starvation and repressed by glucose [9,11,19,20]. However, whereas in *Neurospora* the half-time for inactivation of the repressible system by glucose was 40 min [21], we estimate the half-time for inactivation of the high affinity system in *Coprinus* to be of the order of 5—7 s. This value was determined from experiments in which tissue germinated in acetate-medium was exposed to 25 mM glucose for brief periods of time; at the end of each exposure period the tissue sample was rapidly washed free of glucose and its ability to accumulate [^{14}C]sorbose (initial concentration 3 mM) assessed by a rate assay consisting of five samples taken at 50-s intervals. Measurement of the time required for restoration of the high affinity system on removal of glucose was done in much the same way except that ability to accumulate [^{14}C]sorbose was assessed in samples of glucose-grown tissue after it had been exposed for different periods of time to sugar-free medium. The half-time for restoration of high affinity system activity was 3.5 min. Inclusion of cycloheximide in the starvation medium of this latter experiment had no effect on restoration of high affinity system activity. However, over longer time intervals the high affinity system activity did decay in cells exposed to acetate medium containing cycloheximide. The half-time of this decay was 80 min and this is assumed to be a measure of the half-life of the high affinity system. The measurements of the speed of response to glucose made here were very approximate yet their errors

are likely to lead to overestimation of the half-times rather than underestimation. The speed of inactivation by glucose is likely to be underestimated where the test uses an assay which itself involves incubation in glucose-free medium. Similarly, rate of reactivation following on removal from glucose-medium is likely to be underestimated because of the effect of stored glucogenic materials. Thus, while we acknowledge the deficiencies of the technique employed and that these experiments do not permit discrimination between different mechanisms of inactivation we are confident that glucose inactivates the high affinity system in a matter of seconds and that reactivation occurs in a matter of minutes when glucose is removed. The rapidity of these changes seems to rule out the possibility that protein synthesis/degradation is involved in regulation of these transport systems.

Evidence that a single carrier serves both uptake systems

A wide variety of data demonstrate emphatically that *Coprinus* possesses two glucose transport systems which differ from one another in many respects. However, the rapidity of the regulatory response to glucose implies that the regulation is one which involves activation and inactivation of preformed proteins rather than protein synthesis and degradation. The further implication is that the two uptake systems represent alternate expressions of a single entity. Kinetic data obtained for glucose, 2-deoxy-D-glucose and 3-*O*-methylglucose can be interpreted in a way which is compatible with this view. Lineweaver-Burk plots were biphasic, concave downwards (Fig. 2). In no case did the Hill coefficient derived from such data exceed 0.6. While not diagnostic, these are features of negative co-operativity [22] and along with the regulatory data could be taken to indicate that the high and low affinity activities are alternative phenotypes of a single allosteric protein. The simplest contrasting view is that two proteins are involved in a co-ordinated way; however, genetic data support the single protein interpretation.

Mutants in gene *ftr* are defective in sugar accumulation yet their glycolytic and pentose phosphate pathways are unimpaired, indicating a lesion in sugar transport rather than its metabolism [1]. Alleles of the *ftr* cistron respond differently to different sugars [3], and the gene map positions of mutants obtained in selection experiments depend on the identity of sugars used in selection media [6]; these are features expected only if the gene specifies a protein which interacts directly with the sugar substrate. Of the components likely to be involved in the transport mechanisms detailed above only the carrier (= binding protein) must necessarily interact with all of the different substrates known to be significant in the physiological phenotype of *ftr* mutans. Thus we conclude that the *ftr* cistron is the structural gene for a transport carrier.

Mutants of the *ftr* gene have been selected on media containing up to 5 mM 2-deoxy-D-glucose. On such media the low affinity transport system would be in operation (selection media had NCM as their basis so, apart from the addition of agar, were directly analogous to the reaction media used in transport studies). However, although selection pressure was exerted against the low affinity system the mutants obtained were defective in fructose accumulation despite the fact that transport of this sugar is the responsibility of the high

affinity system [1]. Conversely, mutants selected on media containing sorbose (selection pressure exerted against the high affinity system) were resistant to 2-deoxy-D-glucose at concentrations now known to be far in excess of those required to activate the low affinity system [2]. It seems, therefore, that selection for mutation in one of these uptake systems has inevitably been accompanied by lesion in the other. This conclusion has not been reached by consideration of small, unrepresentative samples. In the course of these studies about 10^9 viable spores were subjected to the various selection procedures; 713 mutants were obtained, all of which proved to be alleles of the *ftr* cistron. A randomly chosen sample of 157 have been positioned in the allele map of this gene.

Preliminary analysis of sugar transport by a few *ftr* alleles seems to confirm these conclusions. Much lessened affinity for glucosamine (prototroph K_m = 0.65 mM, but in mutant Z215 K_m = 135 mM, in Z154 K_m = 7.4 mM, and in Z501 K_m = 13 mM), fructose (in Z154 K_m = 58 mM and in Z501 K_m = 4.2 mM), and sorbose (in Z154 K_m = 5.5 mM and in Z501 K_m = 49 mM) reveals serious damage to the high affinity system. In the same three strains uptake of 2-deoxy-D-glucose by the low affinity system was inhibited by sodium azide (a direct contrast with the prototroph), and although K_m values for glucose and 2-deoxy-D-glucose were little changed the rates of uptake were greatly reduced in the mutants. However, comparison of uptake rates between strains is difficult when those rates are estimated in terms of tissue dry weight without simultaneous assessment of the activity to dry weight relationship. Much more work needs to be done with the mutants before their biochemical phenotype can be considered to be adequately defined. Nevertheless, an extensive range of biochemical and genetic data are in accord with the idea that the *ftr* cistron is the structural gene for a protein which serves both transport systems; discrimination between the two systems depending on negatively co-operative modulation of high affinity carrier activity by the substrate glucose.

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