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SORBOSE TRANSPORT IN *NEUROSPORA CRASSA*

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SUMMARY

1. The transport of sorbose into late log-phase cultures of *Neurospora crassa* does not occur against a concentration gradient, but obeys saturation kinetics, is energy-requiring, and is competitively inhibited by glucose.

2. The apparent K_m for transport is 116 mM, and the maximal velocity is 0.92 μmole/mg dry wt. per h.

3. Adding glucose to cells which have been previously equilibrated with sorbose causes the counterflow of sorbose against a concentration gradient.

INTRODUCTION

That colonial growth can be induced in *Neurospora crassa* by the addition of the ketohexose L-sorbose to the culture medium has been known since the work of TATUM, BARRATT AND CUTTER[1] in 1949. Neurospora hyphae normally grow in the form of long filaments which may attain a length of several cm, and average 3–4 μ in diameter[2]. Septa occur at fairly regular intervals along the hyphae, and some branching is normally seen. Colonial cultures, on both liquid and solid medium, are characterized by a striking increase in the number of branches and septa per unit length of hypha, with growth along the long axis of the hypha severely restricted. When conidia are inoculated onto sorbose agar, they grow in the form of small, discrete colonies, rather than long, branched filaments. In liquid medium one sees discrete, spherical colonies, each one consisting of the germinated mycelia from a single conidium, whereas normally there is a single network of spreading mycelia.

Many colonial mutants of Neurospora have been isolated[3,4] which are the result of mutations at single genetic loci. Some of these mutants are morphologically almost identical to sorbose-induced colonials[5].

The present studies were undertaken to elucidate the mechanism by which sorbose induces colonial growth, which is of considerable importance because of its possible relation to the mechanism of gene-determined morphogenesis. In order to investigate the effects of sorbose on the normal metabolism of the wild type and also of a sorbose-resistant mutant[6], it was first necessary to study the transport of sorbose

into Neurospora mycelia, and the metabolic fate of sorbose itself. This report is concerned with the transport of sorbose into wild-type Neurospora mycelia.

METHODS

Strains and media

Wild-type strain RL38A was used throughout. Stocks were maintained on slants of glycerol–sucrose–complete agar[7].

VOGEL's minimal medium[8] was used for all growth and transport studies. Sterile, 40% stock solutions of sorbose were prepared by treating a hot solution of sorbose with activated charcoal (Darco G-60), filtering, and boiling the colorless filtrate for 5 min. Autoclaving sorbose solutions together with minimal medium resulted in some decomposition, as evidenced by a brownish color, so the stock solution was added to previously autoclaved minimal medium to the desired concentration.

Reagents

L-Sorbose was obtained from Nutritional Biochemicals Corporation. Uniformly ^{14}C-labeled L-sorbose was obtained from Nuclear Chicago Corporation.

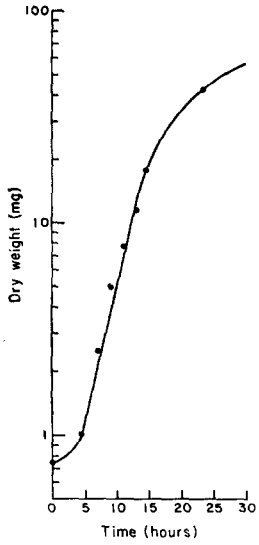
Cultures

Transport studies were carried out with Neurospora mycelia which had been obtained from shake cultures. The advantages of growing Neurospora in shake cultures have been discussed previously[9,10]. In the present study, a suspension of fresh conidia was inoculated into 125-ml erlenmeyer flasks containing 20 ml liquid minimal medium *plus* 1% glucose, and then incubated at 30° on a rotary shaker at 130 rotations/min. At 16–18 h the cultures were harvested on filter paper, washed with distilled water, and resuspended in sterile medium containing substrates for transport studies.

Fig. 1 shows a typical growth curve. After a lag of approx. 4 h, the cells enter an exponential phase, which lasts for 15–16 h. In liquid medium, germination occurs by the extension of hyphae from the conidia, and during the first part of the exponential phase, the hyphae grow primarily by an increase in length, with few branches or septa being formed. Towards the end of the exponential phase, more branching and cross-wall formation occurs. Mycelia were harvested at 16–18 h, in the late log-phase stage of growth, in order to obtain cultures in which all the cells have passed the germination and early growth stages and yet do not vary over too wide a range with respect to age. It is important to use cells at this stage of growth when studying the mechanism by which sorbose induces morphological changes in Neurospora, so as to eliminate any additional effects which sorbose might have on germination and early growth. Late log-phase cells were also used for transport studies so that the results could best be related to future work on the mechanism of action of sorbose.

Measurement of sorbose uptake

Late log-phase cells were harvested, rinsed, and transferred to 125-ml flasks containing 20 ml minimal medium *plus* [^{14}C]sorbose. The cultures were incubated at 30° on a rotary shaker, and harvested at appropriate time intervals. The mycelia



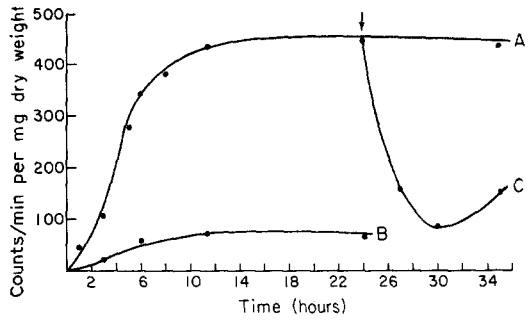


Fig. 1. A typical growth curve for wild-type Neurospora in minimal medium *plus* 1% glucose. Conditions as described in text. Dry weight is plotted on a logarithmic scale.

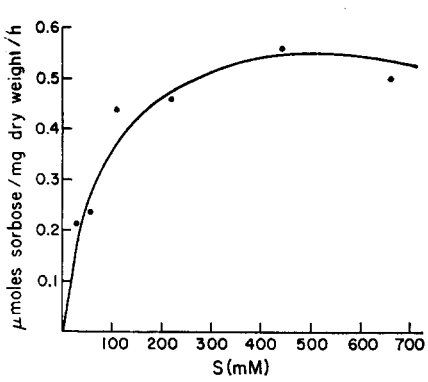
Fig. 2. The uptake of sorbose into Neurospora as a function of time at 30°. Curve A: Medium contains 0.2% glucose *plus* 2.0% sorbose. Curve B: Medium contains 2.0% glucose *plus* 2.0% sorbose. Curve C: Glucose added to the medium at 24 h to a final concn. of 2.0%. Points are averages of 3 determinations.

were harvested on Millipore filters (pore size 1.2 μ), washed 3 times with 60 ml cold distilled water, and transferred to 25-ml erlenmeyer flasks containing 3 ml 75% ethanol. Mycelial extracts were obtained by boiling for 1 min, and removing the extracted mycelia by filtration on a Millipore filter. The mycelia were washed 2 times with 2 ml of 75% ethanol, dried at 80–90°, and weighed. The ethanol extracts and washings were combined and sorbose uptake was determined by measuring the radioactivity of aliquots in a Packard Tri-Carb scintillation counter. Sorbose in the extracts was also measured colorimetrically by the method of DISCHE AND DEVI[11].

For measurements of the rate of sorbose uptake, cultures were harvested after 3 h incubation. At this time, all of the counts which had been removed from the medium were present in the ethanol extracts, and all of the radioactivity in the extracts was in the form of sorbose, as was shown in the following ways: (1) The specific activity of sorbose in the extracts (based on a colorimetric determination of sorbose[11]) was the same as that of the sorbose added to the medium. (2) Paper chromatography of the extracts in 2 solvents, phenol–H_2O (88:12, v/v), and ethyl acetate–pyridine–H_2O (12:5:4, by vol.), revealed only 1 radioactive spot with an R_F identical to sorbose.

RESULTS

When the transport of sorbose into Neurospora mycelia is studied as a function of time, it is seen that the concentration of sugar inside the cell progressively increases until saturation occurs, after which time the intracellular concentration remains constant (Curve A, Fig. 2). At saturation, the intracellular concentration is 0.4 μ-



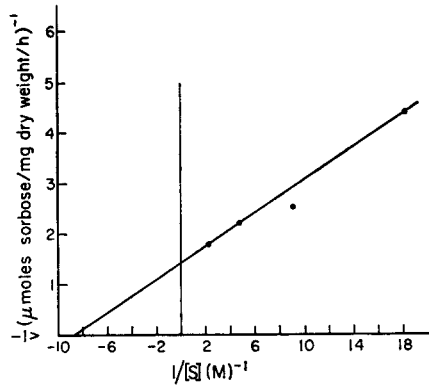


Fig. 3. Rate of sorbose uptake as a function of external concentration. Cultures were harvested after 3 h incubation. Points are averages of 3 determinations.

Fig. 4. A Lineweaver –Burk plot of sorbose uptake data. Cultures were incubated for 3 h at 30°.

moles/ml cell water. (The intracellular water was calculated according to SLAYMAN AND TATUM[12].) The concentration of sorbose in the medium is 0.11 M, or 265 times greater than the intracellular concentration. Sorbose is clearly not transported into Neurospora against a concentration gradient.

Since such a small fraction of the external sorbose is removed from the medium by the cells, transport studies can be carried out for at least 8 h without appreciable alteration of the external concentration.

Fig. 3 shows that the rate of sorbose uptake is concentration dependent and obeys saturation kinetics.

Fig. 4 is a Lineweaver–Burk plot of data obtained during the first 3 h of uptake, while the uptake rate is linear, and the loss of sorbose from the medium is negligible. The apparent K_m for sorbose transport is 116 mM, and the maximal velocity is 0.92 μmoles/mg dry wt. per h.

Sorbose uptake is competitively inhibited by glucose (Table I), and also by mannose, fructose, and 2-deoxyglucose. Glucose not only inhibits sorbose uptake (Curve B, Fig. 2), but if glucose is added to cells previously equilibrated with sorbose,

TABLE I

INHIBITION OF SORBOSE UPTAKE

2.0% sorbose was present in all flasks. Cultures were harvested at 6 h.

Inhibitor	*Concentration*	*Inhibition (%)*
Glucose	0.2%	26
Glucose	0.5%	55
Glucose	1.0%	78
Glucose	2.0%	82
Glucose	4.0%	90
Mannose	2.0%	80
Fructose	2.0%	71
2-Deoxyglucose	2.0%	85
2,4-Dinitrophenol	10^{-4} M	87

sorbose efflux occurs against a concentration gradient (Curve C, Fig. 2). This phenomenon, known as counterflow, has been described by CIRILLO in baker's yeast and Ehrlich ascites cells[13,14].

Sorbose uptake requires energy, as is shown by the inhibition of uptake by 2,4-dinitrophenol (Table I). 2,4-Dinitrophenol markedly reduces O_2 consumption[15], as well as the intracellular level of ATP in Neurospora[16].

DISCUSSION

Sorbose transport has been studied previously in Ehrlich ascites cells[14,17], and in baker's yeast[13,18]. Uptake of sorbose into yeast, ascites cells, and Neurospora exhibits many similarities, and also some differences, as will be discussed below.

(1) Sorbose has been shown not to enter yeast[13], ascites cells[17], or Neurospora against a concentration gradient. Sorbose accumulates in all of these cells, and at equilibrium, when the rate of uptake is constant, the intracellular concentration does not exceed the extracellular concentration. Exit of sorbose against a concentration gradient can be induced in all cases, however, by the addition of glucose to medium containing cells which have been previously equilibrated with sorbose. This phenomenon, known as counterflow, has been cited as evidence that sorbose transport occurs *via* a carrier mechanism, rather than by simple diffusion through membrane pores[13,14].

(2) Michaelis–Menten saturation kinetics have been demonstrated for all 3 types of cells, and the K_m values are reasonably close to one another, 116 mM for Neurospora, 244 mM for yeast[18], and 330 mM for ascites cells[17]. The maximal transport velocity in yeast is 9 μmoles/ml per min (ref. 18) and 11 μmoles/ml per min in ascites cells[17]. In Neurospora, however, the v_{max} is much slower, 0.015 μmole/mg per min. In ascites cells, equilibrium is reached in 1 min at 37° and in 20 min at 20° (ref. 17). In yeast, equilibrium is reached in 60 min at 30° (ref. 13), while in Neurospora it takes 9 h at 30°. It is not clear at the present time why the rate of transport of sorbose into Neurospora should be so much less than into ascites or yeast cells. The v_{max} values for K^+ transport in yeast and Neurospora are in good agreement[19].

(3) In Neurospora, as in yeast and ascites cells, there is competitive inhibition of sorbose transport by glucose. When the external concentrations of sorbose and glucose are equal, glucose inhibits sorbose uptake by 78% in ascites cells[17], and by 82% in Neurospora.

Since the ability of sorbose to induce colonial growth in Neurospora was first demonstrated, it has been realized that the effect could be prevented or reversed by glucose[1]. The basis of this antagonism may simply be that when glucose is present in the medium, less sorbose enters the cells.

(4) Sorbose is not further metabolized in yeast[13], or in ascites cells[14], while in Neurospora it is eventually converted to glucose, with sorbitol as an intermediate, as will be discussed in a later paper.

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