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Regulation of Sugar Transport Systems in *Fusarium oxysporum* var. *lini*

ROGÉLIO L. BRANDÃO† AND MARIA C. LOUREIRO-DIAS*
Laboratory of Microbiology, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal

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***Fusarium oxysporum* var. *lini* (ATCC 10960) formed a facilitated diffusion system for glucose (K_s, about 10 mM) when grown under repressed conditions. Under conditions of derepression, the same system was present together with a high-affinity (K_s, about 40 μM) active system. The maximum velocity of the latter was about 5% of that of the facilitated diffusion system. The high-affinity system was under the control of glucose repression and glucose inactivation. When lactose was the only carbon source in the medium, a facilitated diffusion system for lactose was found (K_s, about 30 mM).**

The understanding of the sugar transport mechanism and its regulation in microorganisms is a fundamental step in the global analysis of its physiology in a habitat where sugar represents a source of energy. Properties of transport systems for sugars in filamentous fungi have been reported in just a few cases. For *Aspergillus nidulans* it was shown that during hexose transport phosphorylation does not occur (4), and the specificity and accumulative properties of the hexose transport systems of this organism were described elsewhere (9). The existence of two transport systems for glucose in *Neurospora crassa* was reported (12, 13). The active transport system was further characterized as an H^+-glucose symport (15).

In recent years, most studies of the regulation of sugar transport in eucaryotic microorganisms have dealt with yeasts. A common feature emerging from the studies performed with yeasts is that whenever a strain displays an H^+-glucose symport, the synthesis of the symport is under the control of glucose repression (7, 8, 10, 16, 19). In the presence of high glucose concentrations, the transport systems found for glucose have in all cases been facilitated diffusion systems. In *Neurospora crassa*, the active transport system for glucose (15) is also under the control of glucose repression.

Fusarium oxysporum was suggested as a producer of protein of high nutritive quality some decades ago (20). In Brazil, where vinasse, a by-product of the distillation of fuel ethanol, represents a serious pollution problem, a major effort to produce this protein is under development. This project is trying to utilize the nutrients present in vinasse as resources for the production of protein. Studies of the nutritive value of the protein produced by using vinasse as a raw material indicate that a high-quality food might be produced when *F. oxysporum* var. *lini* is grown on this substrate (14). The fungus is also able to grow on whey, and the β-galactosidase produced under these conditions has been characterized (3).

The growth parameters of *F. oxysporum* have been studied in some detail, and preliminary studies on sugar transport have been undertaken (2); such studies suggest that under different growth conditions different transport systems are formed. Here we report on some properties of glucose and lactose carriers occurring in *F. oxysporum* var. *lini* cells, describing the conditions under which they are formed and those under which they are inactivated. The ecological advantage of a high-affinity H^+-glucose symport is discussed.

MATERIALS AND METHODS

Organism. *F. oxysporum* var. *lini* ATCC 10960 was kept on potato dextrose agar at 4°C after growth for 7 days at 30°C.

Growth and harvesting conditions. Media used for growth and derepression were previously described (2). For growth and derepression, we used 500 ml of appropriate medium in 2-liter flasks in an orbital incubator at 28°C. Growth occurred for 20 h. The cells were harvested by centrifugation at 20,000 × *g* and washed twice with ice-cold water.

Transport measurements. To measure initial uptake rates of labeled sugars and H^+ movements, we used previously described methods (7). To evaluate the initial uptake rate of labeled sugar, we used about 2.0 mg (dry weight) of biomass in 40 μl of Tris (50 mM) citrate buffer (pH 5). At zero time, 10 μl of a solution of labeled sugar in water was added. Uptake proceeded for 10 s. Control experiments were performed in order to guarantee that the values obtained were true initial values of uptake rate (uptake values were linear for about 20 s in all the concentrations tested). Uptake was arrested by the addition of 5 ml of ice-cold water. All assays were performed in duplicate. D-[1-^{3}H]glucose, [U-^{14}C]lactose, and 3-*O*-methyl-D-[1-^{3}H]glucose were from Amersham Corp. [^{14}C]methyl-β-D-thiogalactopyranoside (TMG) was from Du Pont, NEN Research Products.

Measurement of cellular volume. We used the method described in reference 5, using inulin to define the difference between total and internal volume. We estimated a value of 2.4 μl/mg (dry weight).

Accumulation ratios. Accumulation ratios were calculated for 3-*O*-methyl-D-[1-^{3}H]glucose and for TMG by using specific activities of about 30 and 8 GBq/mol, respectively. The measurements were performed at 28°C in small test tubes with magnetic stirring. The tubes contained 200 μl of cell suspension and 200 μl of 100 mM Tris buffer adjusted to pH 5 with citric acid. At zero time, 100 μl of labeled solution was added to give a final concentration of 1 mM. At the times indicated in the Results, 10-μl samples were taken, filtered, washed, and counted in a liquid scintillation system. Intra-

* Corresponding author.
† Present address: Departamento de Indústria, Escola de Farmácia, Universidade Federal de Ouro Preto, 35400 Ouro Preto, Minas Gerais, Brazil.

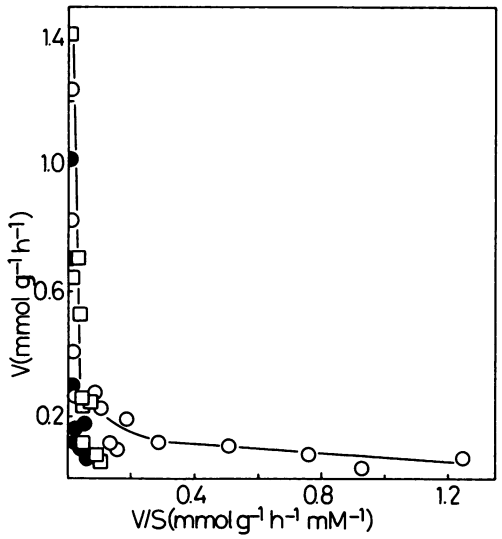

FIG. 1. Eadie-Hofstee plots of the initial rate of transport of [^{3}H]glucose in cells of *F. oxysporum* var. *lini* in repressed (□) and derepressed (○) cells. ●, Values obtained when extrapolated values corresponding to the high-affinity system were subtracted from the experimental values obtained for the higher concentrations in derepressed cells.

cellular concentrations were calculated, with the internal volume indicated above taken into account.

RESULTS

Transport of glucose. Figure 1 represents Eadie-Hofstee plots of the initial uptake rates of [^{3}H]glucose obtained for repressed cells and derepressed cells of *F. oxysporum* in a glucose concentration range of 0.025 to 100 mM. In repressed cells, only one low-affinity transport system was found (or the systems present exhibited similar low affinities). The apparent half-saturation constant (K_s) for this system was about 10 mM, and the maximum velocity (V_{max}) varied between 0.8 and 2 mmol g^{-1} h^{-1}. Derepressed cells clearly exhibited transport systems with two different kinetic properties (open circles in Fig. 1). The high-affinity system (K_s, about 40 μM) had a maximum velocity of about 0.1 mmol g^{-1} h^{-1}. Subtracting this value from the experimental values measured at higher glucose concentrations, we obtained the points represented by closed circles (Fig. 1). It becomes clear that the low-affinity system present under these conditions exhibited the same kinetic characteristics as the one present in repressed cells. In order to elucidate the nature of the two systems, we investigated whether any movement of H^+ became apparent upon the addition of glucose to unbuffered cell suspensions, trying to find evidence for the existence of an H^+-glucose symport. No movements of H^+ were detected. To investigate the abilities of the systems to accumulate substrates, we measured the accumulation ratio of a labeled nonmetabolizable analog of glucose. Previous experiments indicated that 3-*O*-methyl-D-glucose uptake and glucose uptake exhibited mutual competitive inhibition in both types of cells. Figure 2 represents the results obtained for the accumulation ratios under both conditions. The transport system present in repressed cells was facilitated diffusion, while derepressed cells were able to accumulate the analog, which was released by the addition of 10 μm CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) and 1 mM glucose.

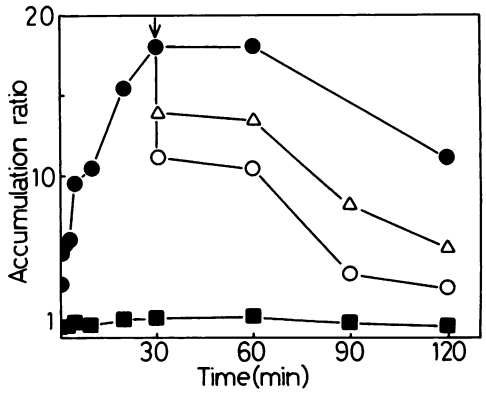

FIG. 2. Accumulation of [^{14}C]3-*O*-methyl-D-glucose (1 mM) by repressed (■) and derepressed (●) cells of *F. oxysporum* var. *lini*. The arrow indicates the addition of 10 μM CCCP (△) or 1 mM glucose (○).

Regulation of glucose transport. In order to monitor the formation of the active system, the kinetics of the uptake rate of [^{3}H]glucose was monitored during the process of derepression. Repressed cells (exponentially growing on 2% [wt/vol] glucose) were subjected to a derepression process (carbon starvation) by incubation in a medium whose carbon source was depleted, followed by the harvesting procedure given in Materials and Methods. The high-affinity system was formed according to the kinetics shown in Fig. 3. After 1 h, the high-affinity system was already apparent and its capacity increased during the next 4 h. This phenomenon was completely abolished by the presence of cycloheximide (20 μg ml^{-1}).

On the other hand, to monitor the fate of the active system when repression conditions were established, derepressed cells were incubated in medium containing 2% (wt/vol) glucose, harvested by centrifugation, and washed twice with ice-cold water. The initial uptake of [^{3}H]glucose in these

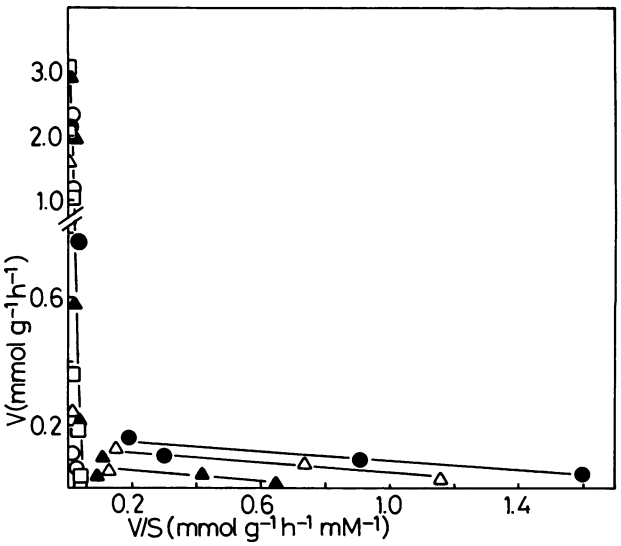

FIG. 3. Formation of the high-affinity transport system for glucose in *F. oxysporum* var. *lini* during starvation. Initial uptake rates of [^{3}H]glucose are represented. Symbols: □, repressed cells; ▲, 1 h after the beginning of starvation; △, 2.5 h; ●, 4 h; ○, 4 h with 70 μM cycloheximide.

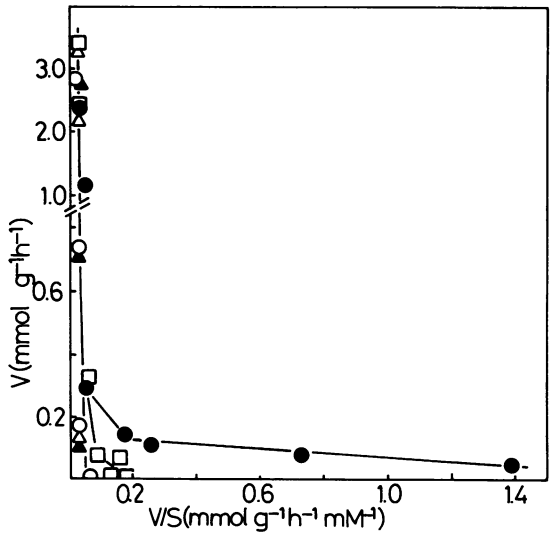

FIG. 4. Loss of activity of the high-affinity transport system for glucose upon addition of 2% (wt/vol) glucose in *F. oxysporum* var. *lini*. Initial uptake rates of [^{3}H]glucose are represented. Symbols: ●, derepressed cells; □, 5 min after addition of glucose; ○, 15 min; △, 60 min; ▲, 60 min with 70 μM cycloheximide.

cells was measured. A rapid loss of activity was noticed (Fig. 4). After 15 min, no uptake was observed through the high-affinity system. The process was independent of the presence of cycloheximide.

Transport of lactose. The measurements of uptake rate of [^{14}C]lactose were performed with a range of concentrations of 10 to 200 mM. The results indicated that a saturable transport system was formed when lactose was the only carbon source in the medium (results not shown). The K_s for lactose was about 30 mM. Starvation of these cells resulted in a loss of activity of the transport system, indicating that the system was inductive. TMG, whose transport exhibited mutual competitive inhibition of lactose transport, was not accumulated in lactose-grown cells, suggesting that the transport system for lactose is a facilitated diffusion system.

DISCUSSION

The results presented above suggest that the transport systems for glucose in *F. oxysporum* var. *lini* are under the same regulation mechanism as those previously described for some eucaryotic microorganisms which display at least one active transport system for glucose (7, 8, 10, 12, 16, 19). A similar situation was described for the high- and low-affinity transport systems for glucose in *Saccharomyces cerevisiae*, although transport occurs by facilitated diffusion in both systems in this yeast (6). It was shown by careful genetic analysis that the high-affinity system in *S. cerevisiae* is under general glucose repression control (1) and that the inactivation of the same system is mediated by a cyclic-AMP-dependent protein kinase (11).

In the fungus discussed in this paper, the active transport system was under the control of glucose repression, and a very fast loss of activity upon glucose addition was observed. It has been found that in some yeasts (16, 19) the two systems may interconvert, with the conversion of the facilitated diffusion form into the active form dependent on de novo protein synthesis. In *F. oxysporum* it is impossible to analyze such phenomena kinetically, since the maximum velocity of the high-affinity system is always very low. Such a low maximum velocity may explain why we could not detect uptake of H^+ together with glucose, although this feature is detectable in some other organisms, in spite of the presence of an active H^+-translocase-ATPase at the plasma membrane. Up to now, accumulative transport of sugars in fungi has been associated with H^+-sugar symports. In the case of *F. oxysporum*, we cannot disregard the hypothesis that some other mechanism is present, but it is also possible that the uptake of H^+ is not detectable because the alkalinization may be very weak and the cell wall may buffer it completely. Such a situation was found in *Candida utilis* (10), but in this yeast the uptake of H^+ became detectable when the yeast was grown in continuous culture under glucose limitation at a low dilution rate. *Fusarium* cells were grown under the same conditions, but no H^+ uptake was detected. One may speculate on the physiological and ecological importance of such a system to the fungus. The system is not synthesized when glucose is freely available, but under starvation conditions, the cells can utilize glucose even at a very low concentration, although only for maintenance purposes since the maximum velocity is too low to allow significant growth. This energy-wasting system loses activity very fast when glucose is available in the environment in appropriate concentrations to enter the cells by the facilitated diffusion system. The difference in affinity between the two transport systems allowed the cells to accumulate 3-*O*-methyl-glucose when the high-affinity system was present, even though the low-affinity system probably represented a leak, but the leak would be significant only at higher sugar concentrations.

A transport system for lactose was previously described for the yeast *Kluyveromyces marxianus* (17). The system was shown to be a H^+-lactose symport able to accumulate TMG. In *F. oxysporum* we could not observe any H^+ uptake together with lactose, nor did the cells accumulate TMG, suggesting a facilitated diffusion system.

In a previous work (2) it was shown that the protonophore carbonyl cyanide *P*-(trifluoromethoxy)-phenylhydrazone (FCCP) inhibits lactose uptake when the fungus cells are previously incubated with the drug. This was interpreted as an indication of lactose being transported by a lactose-H^+ symport. However, it has been shown that uncouplers may interact directly with translocators (18), and for this reason inhibition by protonophores is a poor criterion for establishing the nature of a transport system. Data from the present work favor the hypothesis of a facilitated diffusion system for lactose.

F. oxysporum exhibits some intriguing growth features, such as the ability to acidify the environment when glucose is the carbon source and the ability to raise the pH of the environment when metabolizing lactose (2). These facts were tentatively interpreted with the hypothesis that the nature of the transport systems present in each physiological state would determine the behavior of the fungus. The data we present cannot be easily related with those previous results. Other physiological events, such as the production of acidic compounds or activation of the ATPase by glucose or some metabolite, which are more relevant for such features of fungus growth probably occur.

Studies will proceed for a better elucidation of the sugar transport systems in relation to the physiology of the fungus with the goal of understanding such phenomena as the rapid inactivation that we observed. Also, the mechanism of ATPase activation by the addition of sugars is under investigation.

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