Glucose and Polyol Transport Systems in Candida intermedia and their Regulation

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Transport systems for glucose in the yeast Candida intermedia were compared in cells grown under different conditions. Under conditions of glucose repression the yeast formed a facilitated diffusion system, with a half-saturation constant \((K_m)\) of 2.0 mM. When grown under derepression conditions a \(\text{H}^+\) glucose symport was formed \((K_m 0.16 \text{ mM})\) that was also able to transport L-sorbose and sorbitol \((K_m \text{ about } 200 \text{ mM})\). A \(\text{H}^+\) polyol symport that could transport sorbitol, xylitol, D-arabinitol and mannitol was formed when the cells were grown on sorbitol. This system did not transport glucose. Transport of polyols was not observed when C. intermedia was grown on glucose even when sorbitol was present in the medium.

INTRODUCTION

Different types of transport systems for glucose have been described in yeasts. While in Saccharomyces cerevisiae only facilitated diffusion has been reported (Kotyk & Michaljanková, 1974; Romano, 1982; Franzusoff & Cirillo, 1983). \(\text{H}^+\) glucose symports were described in Rhodotorula glutinis (Höfer & Misra, 1978) and Metschnikowia reukaufii (Alderman & Höfer, 1981).

In Candida wickerhamii both types of systems were found: Spencer-Martins & van Uden (1985) reported that when the yeast was grown on glucose a facilitated diffusion system was formed and when grown on cellobiose a \(\text{H}^+\) glucose symport was formed. In a previous article (Louereiro-Dias, 1987), I presented preliminary data indicating that, in general, yeasts form \(\text{H}^+\)-glucose symports only under derepression conditions.

The transport of polyols in R. glutinis was studied by Klöppel & Höfer (1976a, h). They reported the existence of two transport systems: one low affinity constitutive system and a high affinity inducible system. The constitutive system was shown to be identical to that for monosaccharides. The inducible system was specific for pentitols.

The purpose of this work was to study the transport systems for glucose and other carbon sources, formed under different conditions. Candida intermedia was chosen, for which a large \(\text{H}^+\) uptake together with initial glucose transport has been observed (Louerreiro-Dias, 1987), and which can use a great variety of carbon sources (Meyer et al., 1984). The transport of sorbitol, the polyol most widespread in nature, was studied under conditions of repression, derepression and induction, trying to establish the type of carrier systems involved for both glucose and sorbitol and to understand their regulation.

METHODS

Organism. Candida intermedia strain 2482 from the culture collection of the Gulbenkian Institute of Science was used. Inocula were prepared on slants containing glucose (2.0%, w/v), peptone (1.0%), yeast extract (0.5%), and agar (2.0%, w/v) and were grown for 24-48 h.

Growth and harvesting conditions. Cultures were grown in 1 litre of a mineral medium (van Uden, 1967), with glucose or sorbitol at the concentrations indicated below. At 25°C in an orbital shaker at about 120 r.p.m.: cells were harvested when the OD_{560} of the cultures was about 1. Cultures grown on ethanol were shaken at about 60
Cells were harvested by centrifugation, washed twice with water at 4 °C and resuspended in water at a final suspension density of 30–40 mg dry wt ml⁻¹.

Measurement of initial uptake rates of labelled compounds. The incorporation of D-[1-³H]glucose (for 10 s in duplicate) or [U-¹⁴C]sorbitol (for 5 s in triplicate) was measured as described by Loureiro-Dias & Peinado (1984). Tris/citrate buffer (pH 5.0, 100 mM, 20 µl), 20 µl of cell suspension and 10 µl of a solution of the labelled compound (1000 c.p.m. mmol⁻¹) were incubated at 26 °C for the time indicated. Incorporation was stopped by the addition of 5 ml ice-cold water. Cells were immediately filtered and washed, and radioactivity was counted in a liquid scintillation system.

D-[1-³H]glucose, [U-¹⁴C]sorbose and [U-¹⁴C]sorbitol were purchased from Amersham.

Measurement of cellular volume. The intracellular volume was determined according to Rottenberg (1979) as described by De la Peña et al. (1982) using H₂O [250 µCi ml⁻¹ (9.25 MBq ml⁻¹)] (Amersham) to define the water volume and [¹⁴C]methoxyinulin [6.1 mCi g⁻¹ (225-7 MBq g⁻¹)] (New England Nuclear) to define the extracellular volume. The internal volume varied significantly with the carbon source in the medium: it was 1.97 µl (mg dry wt)⁻¹ for cells grown on glucose (2%, w/v) and 0.89 µl (mg dry wt)⁻¹ for cells grown on ethanol (2%, v/v).

Measurement of initial rates of H⁺ uptake. This was done as described by Loureiro-Dias & Peinado (1984). A pH electrode was attached to a pH meter connected to a recorder through a bucking-voltage device; full scale deflection represented 0.4 pH units. The baseline was obtained with a suspension of cells in water (about 5 mg dry wt ml⁻¹). The addition of a solution of glucose or other compound caused uptake of H⁺, observed as an external alkalinization. The slope of the initial part of the curve was taken as a measurement of proton uptake. The instrument was calibrated with 10 µM HCl. Experiments were done at 26 °C.

Measurement of accumulation ratios. Accumulation ratios were calculated for L-[U-¹⁴C]sorbose and [U-¹⁴C]sorbitol, using specific activities of about 2 Ci mol⁻¹ (74 GBq mol⁻¹). The measurements were done at 26 °C in small test-tubes with magnetic stirring. Tubes contained 200 µl of cell suspension and 200 µl Tris/citrate buffer, pH 5.0. At zero time, 100 µl of labelled solution were added to give a final concentration of about 1 mM. During 2 h, 10 µl samples were taken, filtered and washed, and radioactivity was counted in a liquid scintillation system. Intracellular concentrations were then calculated taking into account the cellular volumes measured above. When indicated carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added (as a 200 mM solution in ethanol).

RESULTS

Transport systems for glucose

In order to compare transport systems under different physiological conditions, cell suspensions of yeast were prepared under repression conditions (exponential phase, 2% w/v glucose) and derepression conditions (2% v/v, ethanol), and external alkalinization during the initial uptake of glucose was measured (Fig. 1). While in ethanol-grown cells there was an immediate, large H⁺ uptake, no corresponding external alkalinization was observed for glucose-grown cells. The external acidification observed in this case, which probably involves the activation of a H⁺-pump, also occurred in ethanol-grown cells after about 20 s (not shown).

In both type of cells the initial rate of transport of [³H]glucose was measured. From these values, Lineweaver–Burk plots were made and half-saturation constants (Kₘ) were calculated to be 0.16 mM for the transport system present in derepressed cells and 2.0 mM for repressed cells. The relative Vรมax was 2.0 mmol g⁻¹ h⁻¹ in both cases. A comparison between initial rates of uptake of [³H]glucose and H⁺, calculated for different glucose concentrations in the Kₘ range, indicated that about 2 mol H⁺ were taken up per mol of glucose in cells grown on ethanol. In these cells, D-galactose, L-sorbose, D-xyllose and 3-methyl-D-glucose competitively inhibited the transport of [³H]glucose (not shown).

The nature of the transport systems in both types of cell was further studied by measuring their ability to concentrate an analogue of glucose that was not readily metabolized. In previous experiments, mutual competitive inhibition of transport of L-sorbose and glucose was observed in both types of cell, which indicated that both sugars used the same transport systems. For cells incubated in the presence of 1 mM-L-[U-¹⁴C]sorbose, values for the intracellular concentrations and the accumulation ratios were calculated (Fig. 2). In cells grown on glucose, the concentration was about the same on both sides of the plasma membrane, while ethanol-grown cells showed CCCP-sensitive accumulation of sorbose. In both cases the addition of glucose induced counterflow of L-sorbose.
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**Transport systems for sorbitol**

To establish the kinetics of sorbitol transport in sorbitol-grown cells, the initial uptake rate of [U-14C]sorbitol in the range 1.5–15 mM was measured in the presence or absence of other polyols. Xyitol (20 mM) inhibited the transport of sorbitol but an increase in concentration did not induce further inhibition (Fig. 3). To explain this unusual result the hypothesis was raised that sorbitol might be transported by two systems: one inhibited completely by the concentrations of xyitol used but the other not inhibited at all. Typically, the transport system which was left after xyitol inhibition showed a higher $V_{\text{max}}$ than before. Values of initial uptake of [U-14C]sorbitol in the range 3–200 mM (Fig. 4) indicated the existence of two transport systems, with $K_m$ values of about 6 mM and 200 mM. In order to elucidate the nature of these transport systems the cells were grown on different carbon sources, in an attempt to create conditions under which only one transport system would be present.

*Glucose-grown cells.* When the cells were grown on glucose (2%, w/v) no [14C]sorbitol uptake was detected at concentrations up to 200 mM. No external alkalinization of the external environment was detected when sorbitol was added to an unbuffered cell suspension. Neither sorbitol (100 mM) nor glucose (100 mM) inhibited [3H]glucose transport (results not shown), implying that sorbitol did not use the facilitated diffusion system for glucose.

Identical results were obtained with cells grown on glucose (2%, w/v) plus glucose (4%, w/v), indicating that glucose repressed the synthesis of both transport systems for sorbitol.

*Ethanol-grown cells.* In cells grown on ethanol (2%, v/v), the initial uptake kinetics of [U-14C]sorbitol showed that there was only one low affinity system, which was competitively inhibited by 0.5 mM-glucose. In the presence of 5 mM-glucose no uptake was detected. Sorbitol at concentrations of 0.5 and 1 M competitively inhibited [3H]glucose transport (results not shown). Accumulation of [14C]sorbitol by ethanol-grown cells was CCCP sensitive. Glucose (100 mM) or sorbitol (100 mM) induced an immediate counterflow of [14C]sorbitol (Fig. 5).

*Sorbitol-grown cells.* Two transport systems for sorbitol were found in cells grown on sorbitol (2%, w/v). An Eadie–Hofstee plot of glucose inhibition of sorbitol transport is shown in Fig. 6. To show which transport system was inhibited by glucose, we tried to separate the values of the
initial rates concerning each system by using a numerical iterative method. From a Lineweaver-Burk plot for each system it became clear that glucose did not inhibit the high affinity system \( K_m = 6.2 \text{ mM} \) and \( V_{max} = 0.8 \text{ mmol (g dry wt)}^{-1} \text{ h}^{-1} \) for all the concentrations of glucose indicated in Fig. 6 but did inhibit the low affinity system.

In sorbitol-grown cells addition of sorbitol to unbuffered cell suspensions produced an external alkalization with a stoichiometry of 1 mol H\(^+\) taken up per mol of sorbitol. A strong
alkalinization was also observed when xylitol, D-arabinitol and mannitol were added to sorbitol-grown cells, but ribitol, L-arabinitol, erythritol, inositol and galactitol produced no alkalinization. This may indicate that xylitol, D-arabinitol and mannitol use the same transport system as sorbitol.

To study the ability to accumulate sorbitol, cells were grown with other polyols as carbon source, to see whether free sorbitol could be accumulated by an active process. Although there was accumulation of [14C]sorbitol, counterflow could not be induced. Possibly sorbitol within the cells was quickly oxidized by a polyol dehydrogenase (Barnett, 1968). The low specificity of such enzymes for polyols was reported for other species of the genus Candida by Chakravorty & Horecker (1966).

**DISCUSSION**

The results clearly showed that the carbon source present in the growth medium determined which transport systems were formed in cells of C. intermedia. When glucose was present at high concentrations a low affinity facilitated diffusion system for glucose was formed. When the cells were growing in the absence of glucose (ethanol-grown cells), a H+-glucose symport was formed. This means that the latter cells could utilize any trace of glucose present in the environment, by means of a high affinity accumulative transport system. The same transport system was formed when glucose-grown cells were starved for 2 h in a medium without carbon source: its synthesis was sensitive to the presence of 0.1% cycloheximide, indicating that a new protein was formed under such conditions (results not shown).

Sorbitol transport fell into three categories. No uptake of sorbitol could be detected in cells grown on glucose, indicating that neither a transport system specific for sorbitol was synthesized nor was sorbitol transported by the facilitated diffusion system for glucose. In ethanol-grown cells, sorbitol could be transported by the H+-glucose symport. This system was able to transport several sugars, but showed a low affinity for sorbitol. When sorbitol was present in the medium in the absence of glucose the cells synthesized a H+-polyol symport, specific for polyols, that was not used by glucose.

The transport of polyols in C. intermedia resembles the transport of pentitols described by Klöppel & Höfer (1976a, b) for R. glutinis. These authors reported that glucose inhibited the polyol specific system non-competitively, but their measurements did not represent initial rates of transport. In the present work the uptake of sorbitol in the presence of glucose was linear for only a few seconds: 10 s was too long for measurements of initial rates of transport. Another difference concerns specificity, since in R. glutinis sorbitol was not transported.

Future work in this field will involve the isolation of mutants of C. intermedia lacking the H+-glucose symport. Such mutants should transport sorbitol through the H+-polyol symport and it will thus be possible to study this transport system without the interference of the H+-glucose symport.

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**REFERENCES**


