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Kinetic analysis of hexose uptake in *Saccharomyces cerevisiae* cultivated in continuous culture

Michelle M.C. Meijer [a,*], Johannes Boonstra [a], Arie J. Verkleij [a], C. Theo Verrips [a,b]

[a] *Department of Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands*
[b] *Unilever Research Laboratory, Olivier van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands*

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Abstract

As the expression of the hexose transporters in *Saccharomyces cerevisiae* is regulated by the external fructose concentration, in vivo fructose transport was investigated at a number of different but constant fructose concentrations in a continuous culture. The in vitro uptake rate and kinetic parameters were studied by uptake of ^{14}C-labelled fructose. From Eadie–Hofstee plots, based on the results of the in vitro uptake experiment, K_m and V_{max} for the different steady-state conditions were determined. These results demonstrate that as fructose concentrations increase, fructose consumption rates and K_m increase and V_{max} slightly decreases. However, remarkably a large discrepancy was observed between in vivo determined consumption rates in the continuous culture and the in vitro uptake rates from the ^{14}C-labelled fructose uptake experiment on cells from an identical steady-state condition. In every condition the in vivo consumption rate was approx. 2–3 times higher than the in vitro uptake rate. A comparable discrepancy was observed for glucose uptake rates; however, in vivo and in vitro galactose uptake rates were identical. These observations imply that kinetic analyses of sugar transport mechanisms can only be performed correctly when accompanied by a comparison with in vivo determined consumption rates.

Keywords: *Saccharomyces cerevisiae*; Carbon metabolism; Hexose uptake; Continuous culture

1. Introduction

The yeast *Saccharomyces cerevisiae* is able to grow on a variety of sugars which serve as carbon- and energy source. The first step in hexose consumption concerns the transport of the hexose molecule across the plasma membrane by a facilitated diffusion system. Until recently, it was assumed, based upon kinetic studies from 5-s uptake experiments, that the hexose transport in *S. cerevisiae* was mediated by two kinetically distinct hexose transport systems, i.e., a high affinity transport system (K_m of 1–2 mM and 5–10 mM for glucose and fructose respectively) and a low affinity transport system (K_m of 20 mM and 50 mM for glucose and fructose respectively) [1,2].

Recently, however, evidence has been obtained that hexose transport in *S. cerevisiae* is mediated by multiple, differentially regulated hexose transporters [3–6]. These transporters are encoded by a multigene family of genes, i.e., the *HXT*-genes, *GAL2* and *SNF3*. The expression of the various members of this family is differentially regulated dependent upon the external glucose concentration [7]. Moreover, some members of the transporters may influence the activ-

* Corresponding author: Fax: +31 30 2513655; e-mail: michelle@emsaserv.biol.ruu.nl

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ity of others. In addition to the differential expression, the hexose transporters exhibit different specificities for glucose, fructose and mannose. These observations clearly indicate that hexose transport in *S. cerevisiae* is mediated by a complex network of transport systems, the activity being strongly determined by the external hexose concentration. Consequently, a kinetic analysis of hexose transport in wild-type *S. cerevisiae*, grown in batch culture may yield results that would be difficult to interpret, because of the varying external hexose concentrations.

In this respect the application of a continuous culture of yeast cells may provide the required conditions for accurate hexose transport studies. We demonstrate here that growth of *S. cerevisiae* in a nitrogen-limited continuous culture in the presence of various concentrations of fructose in the feed medium provides the possibility to grow the cells under physiological well-defined conditions in the presence of a number of different but constant fructose concentrations. Using these cells we demonstrate that fructose transport can be described according to the kinetics of a 'one-component' system under each tested condition. Increasing the fructose concentration in the feed medium is accompanied by an increase in fructose concentration in the growth medium which results in a decrease of the V_{max} and an increase of K_m. However, at all fructose concentrations used, the in vivo fructose flux, deduced from the reliable continuous culture experiments, appears to be approx. 2- to 3-fold higher than the calculated flux obtained from in vitro uptake experiments. This difference was found also for glucose flux. No difference, however, was observed between in vivo and in vitro galactose influx.

2. Materials and methods

2.1. Reagents

D-[U-^{14}C]-fructose, D-[U-^{14}C]-glucose and D-[U-^{14}C]-galactose were obtained from Amersham Life Science Nederland B.V. ('s-Hertogenbosch, The Netherlands). Chemicals were purchased from E. Merck Nederland B.V. (Amsterdam, The Netherlands) or Sigma Chemical Co. (Zwijndrecht, The Netherlands) and were reagent grade or better.

2.2. Strain and growth conditions

Saccharomyces cerevisiae strain SU32 was grown in a 2 l BiofloIII chemostat (New Brunswick Scientific; Nijmegen, The Netherlands), that was connected to a computer controller unit running on Advanced Fermentation Software (New Brunswick Scientific). SU32 was inoculated in the medium as described previously [8] and after batch growth overnight a continuous feed was connected. The medium for continuous cultivation is as previously described [8] with the modification that the NH_4^+ concentration was 1.5 $g \cdot l^{-1}$ and the fructose feed concentration was changed for each steady-state. At a dilution rate of 0.3 h^{-1} a steady-state analysis was performed at fructose feed concentrations of 121, 147, 175, 201, 264, 309, 434, 600 and 720 mM.

In order to perform the glucose and galactose uptake experiments, SU32 was cultivated in the medium described above except for the modification that the concentration glucose or galactose in the feed was 167 mM and the dilution rate was 0.13 h^{-1}. The NH_4^+ concentration was 1.5 $g \cdot l^{-1}$.

The pH of the cultures was automatically controlled at 5 by the addition of 5 M KOH. Temperature was kept at 30°C. The airflow and stirrer speed were 2 $l \cdot min^{-1}$ and 700 rpm respectively. Hereby the oxygen tension was kept above 50%. Carbon dioxide production (rCO_2 in mmol · (g dry weight)$^{-1}$ · h^{-1}) and oxygen consumption (qO_2 in mmol · g^{-1} · h^{-1}) were measured on line and continuously by connection of the headspace of the chemostat to an Ura3G CO_2 analyser and a Magnos4G O_2 analyser (Hartmann and Braun).

Samples for the determination of external sugar concentrations and gram dry mass per litre culture liquid (abbreviated as g) were taken from the chemostat under steady-state conditions and prepared or determined as described previously [8].

2.3. Determination of the in vivo sugar uptake rate in the chemostat

In order to determine the fructose, glucose or galactose uptake rate in the chemostat (as from here cited as in vivo sugar uptake rate) the following parameters were measured: dry weight (DW in g · l^{-1}), dilution rate (D in h^{-1}), sugar concentration in

the feed medium (S_{feed} in mM) and the external sugar concentration in the continuous culture ($S_{external}$ in mM). The in vivo sugar consumption rate (in mmol · $g^{-1} \cdot h^{-1}$) is subsequently defined as follows:

$$\Phi_{in\ vivo} = (\{S_{feed} - S_{external}\} \cdot D)/DW \quad (1)$$

2.4. ^{14}C-labelled fructose, glucose and galactose uptake assays

Uptake experiments were performed according to Walsh et al. [9] with some modifications. Continuously grown cells, harvested from the established steady-state in the chemostat were washed and filtered (Whatman G/C glass fibre filters) three times and resuspended in ice-cold 100 mM potassium phosphate buffer (pH 6.5) to an optical density of OD_{600nm} = 15. Uptake was measured at fructose concentrations of 1 to 270 mM (specific radioactivity 1 to 74 kBq · μmol^{-1}), at glucose concentrations of 0.3 to 250 mM (specific radioactivity 1 to 248 kBq · μmol^{-1}) and at galactose concentrations of 0.3 to 150 mM (specific radioactivity 1 to 248 kBq · μmol^{-1}). Yeast cells (100 μl) and two-times-concentrated ^{14}C-labelled fructose, glucose or galactose (100 μl) were preincubated under aeration at the assay temperature (30°C). Uptake was started by addition of the ^{14}C-labelled sugar to the cells. After exactly 5 s of incubation the reaction was terminated by quenching the cells in 10 vols. of 100 mM potassium phosphate buffer (pH 6.5) containing 500 mM fructose, glucose or galactose maintained at a temperature below −5°C on salt-ice mixtures. Cells were then collected rapidly and rinsed on glass fibre filters (d = 1 cm) with 10 ml of quenching solution. Filters were then transferred immediately to scintillation vials containing 2.5 ml of AquaLuma scintillant (Lumac.LCD), and radioactivity was measured with a Beckman liquid scintillation counter. The control blank in each experiment was performed as previously described [9]. Each uptake experiment was performed in triplicate. From these uptake experiments the (as from here cited as) in vitro sugar flux (in mmol · $g^{-1} \cdot h^{-1}$) can be calculated according to:

$$\Phi_{in\ vitro} = (S/(S + K_m)) \cdot V_{max} \quad (2)$$

where S (in mM) is the external sugar concentration, K_m (in mM) is the affinity constant and V_{max} (in mmol · $g^{-1} \cdot h^{-1}$) is the maximum rate of sugar uptake. Eadie–Hofstee plots were used to determine the kinetic parameters K_m and V_{max}. All data were analysed by computer-assisted non-linear regression by using Enzfitter software, using proportional weighting.

3. Results

3.1. Growth of S. cerevisiae under constant sugar concentrations

As described above, expression of hexose transporters in *S. cerevisiae* is strongly dependent upon the external hexose concentration, and consequently growth of wild-type *S. cerevisiae* in batch cultures results in continuous changes in expression of different hexose transporters. In order to obtain constant growth conditions of *S. cerevisiae* in this respect, the cells were grown in continuous culture under nitrogen limitation at a dilution rate of 0.3 h^{-1}. The fructose concentration in the feed medium was increased from 121 mM to 720 mM. Under steady-state conditions a constant biomass was obtained of approx. 4.5 g dry weight · l^{-1}, independently of the fructose concentration in the feed medium. These observations demonstrate the nitrogen-limited nature of the culture. The latter was supported by the observations that cells obtained a pseudo hyphae morphology [10,11], especially at the high fructose concentrations. However, at low fructose concentrations, i.e., fructose concentrations that are below the K_m of the fructose uptake system, it is possible that the growth rate is not determined by nitrogen only, as the limiting substrate, but also by fructose. So at low fructose concentrations the cells may be under dual-substrate control [12,13]. However, this will not disturb the steady-state conditions or the application of growing cells under constant fructose concentrations, as is demonstrated below.

The external fructose concentration in the growth medium was determined in the culture medium under steady-state conditions, and as shown in Fig. 1 this concentration increased from 3 mM to 417 mM, as the fructose concentration in the feed medium was increased from 121 mM to 720 mM. Especially at fructose concentrations in the feed medium higher

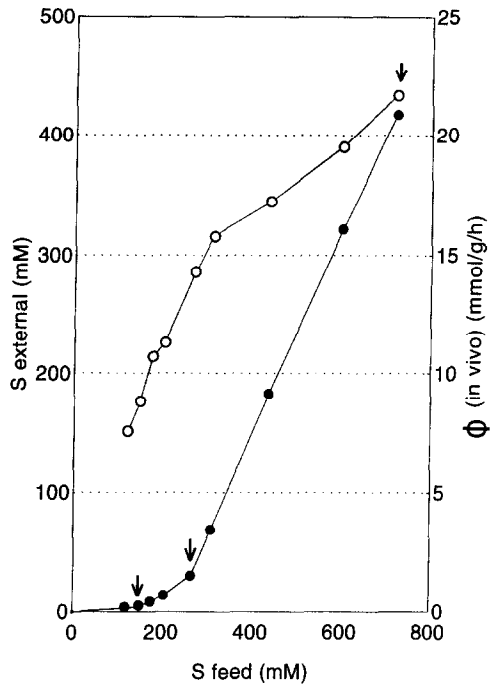

Fig. 1. The external fructose concentration and the in vivo fructose flux Φ as a function of the fructose feed concentration. Yeast cells were cultivated in a nitrogen-limited continuous culture under step-wise increasing fructose concentrations in the feed medium. At each fructose concentration in the feed medium the external fructose concentration (●) (mM) in the chemostat was determined and fructose flux (○) (mmol·g^{-1}·h^{-1}) was determined according to Eq. (1) as described in Section 2. Three of these conditions are chosen to investigate the kinetic characteristics of fructose uptake of cells that are exposed to a low, medium and high fructose concentration (indicated by arrows (↓)).

than 250 mM, a strong increase in the external fructose concentration in the culture medium was observed (Fig. 1). While the fructose concentrations in the feed medium increase the in vivo fructose consumption rates also increase from 7.55 to 21.69 mmol $\cdot g^{-1} \cdot h^{-1}$ (Fig. 1). Error estimations in in vivo consumption rates were always less than 10%. Since the biomass was constant at all fructose concentrations used, these data indicate that fructose metabolism changes at increasing fructose concentrations. This was reflected by an increase in CO_2, ethanol and glycerol production (data not shown).

These observations clearly demonstrate that with help from the continuous culture conditions can be provided under which *S. cerevisiae* can be grown in the presence of a number of different but constant fructose concentrations. Furthermore, the fructose concentration can be modulated easily by adjusting the concentration in the feed medium.

3.2. Fructose uptake

In order to study the kinetics of fructose uptake in cells grown in the presence of various constant fructose concentrations, cells were harvested from the continuous culture, washed and incubated in the presence of ^{14}C-labelled fructose as described in Section 2. The fructose uptake was linear for at least 8 s (data not shown), and therefore a 5-s pulse was used to analyse the dependency of the fructose uptake rate on the fructose concentration as described in Section 2. Determining the fructose uptake rate in relation to the fructose concentration in cells grown either under low (4 mM), medium (31 mM) and high (417 mM) external fructose concentrations, and subsequent expression in an Eadie–Hofstee plot as shown in Fig. 2, reveals in all three cases an apparent linear relationship. These findings indicate that under these conditions fructose transport in *S. cerevisiae* can be described according to the kinetics of a 'one-component' transport system. Subsequently the apparent

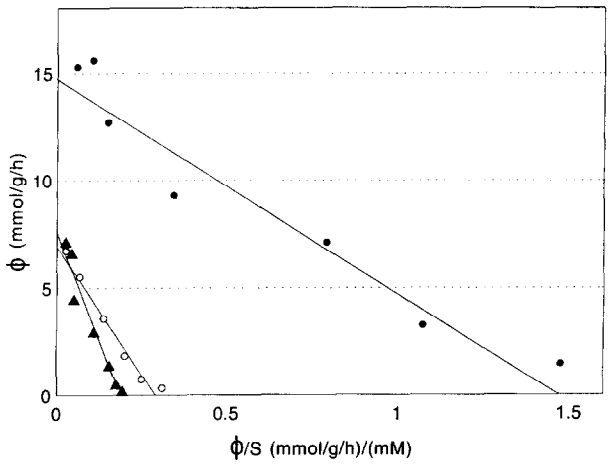

Fig. 2. Kinetics of ^{14}C-labelled fructose uptake of cells grown on low, medium and high fructose concentration in the continuous culture. Fructose uptake was determined of cells grown on a low (4 mM) (●), medium (31 mM) (○) and high (417 mM) (▲) fructose concentration as described in Section 2. Each point is the mean of three determinations. Data are presented in Eadie–Hofstee plot and analysed by using computer-assisted non-linear regression, using proportional weighting. The data were best-fitted to a model of a one-component uptake system.

Table 1
Comparison of in vivo fructose fluxes from cells growing in continuous culture and in vitro fructose fluxes determined from kinetic parameters, K_m and V_{max}, from ^{14}C-labelled fructose uptake experiments

	$S_{external} = 4$ mM	$S_{external} = 31$ mM	$S_{external} = 417$ mM
$\Phi_{in\ vivo}$ (mmol · g^{-1} · h^{-1})	8.8	14.0	21.7
V_{max} (mmol · g^{-1} · h^{-1})	14.7 ± 0.9	6.9 ± 0.4	7.5 ± 0.6
K_m (mM)	10.0 ± 1.3	23.8 ± 2.2	39.6 ± 4.6
$\Phi_{in\ vitro}$ (mmol · g^{-1} · h^{-1})	4.2	3.9	6.7
$\Phi_{in\ vivo}/\Phi_{in\ vitro}$	2.1	3.6	3.2

In vivo fructose fluxes were determined from cells grown in continuous culture on low (4 mM), medium (31 mM) and high (417 mM) fructose concentrations according to Eq. (1) as described in Section 2. In vitro fructose fluxes of these cells were determined after ^{14}C-labelled fructose uptake experiments as described in Section 2 according to Eq. (2).

K_m and apparent V_{max} values were calculated as shown in Table 1. Increasing the external fructose concentration in the growth medium from 4 to 417 mM resulted in an increase of the apparent K_m from 10.0 ± 1.3 to 39.6 ± 4.6 mM and a decrease in apparent V_{max} from 14.7 ± 0.9 to 7.5 ± 0.6 mmol · g^{-1} · h^{-1}.

In order to establish whether these transport parameters represent reliable data, we have calculated the in vivo flux rates from the steady-state continuous culture data and compared these with the in vitro flux data, calculated from the apparent K_m and V_{max} values. The in vivo flux rates were determined from the dilution rate of the continuous culture and the fructose concentrations in feed and culture medium as described in Section 2 (Eq. (1)). These calculations yielded fructose influx rates of respectively 8.8, 14.0 and 21.7 mmol · g^{-1} · h^{-1} fructose in cells grown in the presence of 4, 31 and 417 mM fructose respectively (Table 1). Subsequently the fructose fluxes were calculated from the apparent K_m and V_{max} values (Eq. (2)) and yielded respectively 4.2, 3.9 and 6.7 mmol · g^{-1} · h^{-1} fructose. Clearly the in vitro obtained data are approx. 3-fold lower than the in vivo data (Table 1). These findings indicate strongly that a relevant transport component is lost during the in vitro uptake experiment.

3.3. Glucose transport

Since fructose and glucose are transported by the same transporters, we determined also the in vitro and in vivo glucose flux rates to exclude any possible fructose-dependent artefact of the uptake assays as described above. *S. cerevisiae* was grown in a nitrogen-limited continuous culture with glucose as sole carbon- and energy source using a dilution rate of 0.13 h^{-1}. The glucose concentration in the feed medium was 167 mM. At steady-state conditions the glucose concentration in the culture medium was determined as 0.60 mM. From these data the in vivo glucose flux rate was calculated as 3.2 mmol · g^{-1} · h^{-1}. Subsequently, cells were taken from the continuous culture under steady-state conditions, and used for a ^{14}C-labelled glucose uptake experiment as described in Section 2. ^{14}C-labelled glucose uptake was

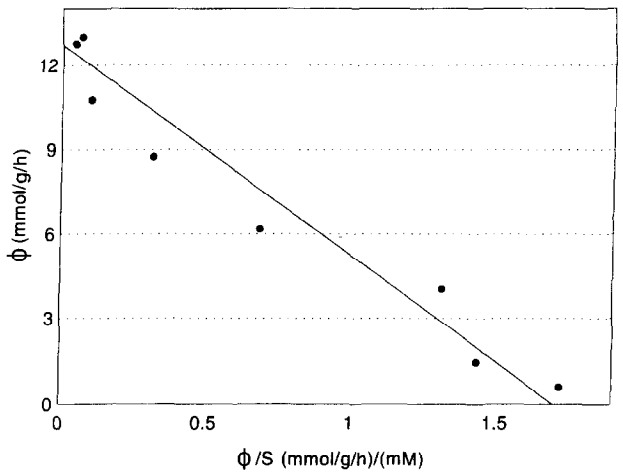

Fig. 3. Kinetics of ^{14}C-labelled glucose uptake of continuously grown cells on 0.6 mM glucose. Glucose uptake was determined as described in Section 2. Each point is the mean of three determinations. Data are presented in Eadie–Hofstee plot and analysed by using computer-assisted non-linear regression, using proportional weighting. The data were best fitted to a model of a one-component uptake system.

linear for at least 8 s, as also found for fructose uptake, and therefore a 5-s pulse was used to analyse the glucose uptake kinetics as described in Section 2. Also for glucose a linear relationship between the glucose flux rate per glucose concentration and the glucose concentration was obtained when plotted in an Eadie–Hofstee plot (Fig. 3). The apparent K_m was calculated to be 7.4 ± 0.7 mM and the apparent V_{max} as 12.7 ± 1.4 mmol · g^{-1} · h^{-1}. From these data the in vitro glucose flux was calculated as 0.95 mmol · g^{-1} · h^{-1}. Thus also for glucose the in vivo uptake rate appears to be 3-fold higher than the in vitro determined influx rate, as was shown to be the case for fructose as well. These data are consistent with a previously described discrepancy between glucose transport and glucose consumption [14].

3.4. Galactose transport

The observed differences between the in vivo and in vitro fructose and glucose flux rates may be caused by the complexity of the transport systems involved. Therefore a similar experiment was performed using galactose, since galactose has been demonstrated to be taken up by the cells by a single transporter, encoded by *GAL2* [15]. Cells were cultured in a nitrogen-limited continuous culture with galactose as single carbon- and energy source at a dilution rate of 0.13 h^{-1}. The galactose concentration in the feed medium was 167 mM, the concentration in the culture medium was determined as 1.98 mM. From these data the in vivo galactose influx rate was calculated to be 3.13 mmol · g^{-1} · h^{-1}. Subsequently the in vitro galactose influx rate was determined from an uptake experiment using ^{14}C-galactose (Fig. 4). The apparent K_m and V_{max} were calculated to be 4.0 ± 0.4 mM and 9.0 ± 0.9 mmol · g^{-1} · h^{-1} respectively. These data yielded an in vitro galactose influx rate of 3.02 mmol · g^{-1} · h^{-1}. Thus for galactose the in vivo and in vitro influx rates were identical, demonstrating that the differences observed for fructose and glucose are likely due to the transport systems involved.

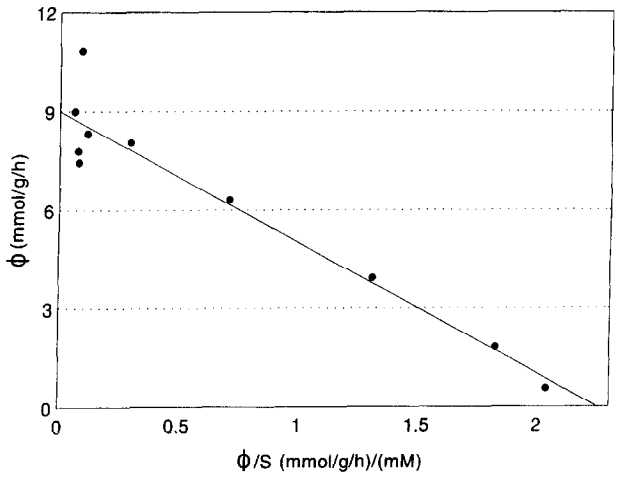

Fig. 4. Kinetics of ^{14}C-labelled galactose uptake of continuously grown cells on 2 mM galactose. Galactose uptake was determined as described in Section 2. Each point is the mean of three determinations. Data are presented in Eadie–Hofstee plot and analysed by using computer-assisted non-linear regression, using proportional weighting. The data were best fitted to a model of a one-component uptake system.

4. Discussion

The existence of a multigene family hexose transporters in *S. cerevisiae* may be essential to adjust the cells to extreme conditions. For instance, in *S. cerevisiae*'s natural habitat of grapefruit juice, the organism is easily exposed to sugar concentrations of 1–3 M, whereas on the other hand it is able to grow on very low sugar concentrations as well. The hexose uptake system might consist of a fine-tuning apparatus to adapt to changing extracellular conditions, in which depending on these conditions different transporter proteins do or do not contribute to the sugar uptake process. As one starts to investigate hexose uptake in such an organism, it is important to consider these conditions, such as the external hexose concentration. Different research groups have shown that external glucose concentration determines the expression and activity of hexose transporters [6,7,16]. In our study on hexose transport we have tried to eliminate the varying external conditions by growing wild-type yeast cells under physiologically defined conditions in a nitrogen-limited continuous culture, where should be noted that at low sugar concentrations growth rate could be under dual-substrate control [12,13]. However, we have shown that under these conditions it is possible to modulate the external sugar concentration by a step-wise increase of the sugar concentration in the feed medium. Subse-

quently three different conditions were chosen to investigate the kinetic parameters of cells that are exposed to either a high-, a low- or an intermediate fructose concentration. Eadie–Hofstee plots based on the results of these uptake experiments are linear in all three cases and therefore under these conditions fructose transport in *S. cerevisiae* can be described according to the kinetics of a 'one-component' transport system. As the external fructose concentration increases, the affinity decreases which is seen by an increase in the apparent K_m from 10.0 to 39.6 mM, which is in correspondence with published data on fructose uptake in batch cultures on low and high fructose [2]. Apart from a decrease in affinity, the increase in external fructose concentration is accompanied by a decrease in the apparent V_{max}, indicating that the activity or the number of active transporters in the membrane decreases. These findings are in agreement with the recent findings of Özcan and Johnston [7].

With the K_m and V_{max} data, the in vitro flux under these three conditions of different constant external fructose concentrations can be calculated. However, when the in vivo and in vitro fructose fluxes are compared, the former fluxes are approx. 2–3 times higher than the latter under each condition. Identical experiments performed on galactose and glucose demonstrate that for Hxt-mediated fructose or glucose transport, one or several essential components are not detectable in ^{14}C-labelled fructose- or glucose uptake experiments. The missing component is independent of the sugar influx and independent of the presence of any medium components that could be lost after washing the cells (data not shown). It is difficult to speculate whether the missing component is independent of the growth rate, while at sugar concentrations below the K_m of their respective uptake system, the growth rate might, besides by the limiting substrate nitrogen, also be controlled by carbon as a substrate [12,13]. The missing component in the transport assay could be caused by a decreased contribution of one particular uptake component, which would be inactivated because under the tested condition. This seems unlikely though because the difference is observed under all conditions that have been studied. More likely would be the inactivation of a general component or a regulator of the uptake system.

The missing component seems to be inactivated very rapidly: the elapsed time between sampling and performing the actual uptake experiment is less than a minute. A fast (de)phosphorylation may explain this quick inactivation. As Vojtek et al. [17], Kriegel et al. [18] and Herrero [19] have described, hexokinase PII has been shown to have a protein kinase activity capable of autophosphorylation and of phosphorylation of other proteins. This protein phosphorylation capacity has been shown to have a substrate specificity similar to a casein-kinase-II-like protein kinase. It is known that all of the Hxt-protein sequences as well as the Snf3-carboxyl terminus contain possible consensus sites for protein phosphorylation, among which several casein-kinase-II-like consensus sites [20]. A possible mechanism could involve a rapid (de)phosphorylation of the Hxt's by hexokinase PII as cells are taken from the chemostat, hereby inactivating part of the uptake mechanism itself or a regulating component of the hexose transporters in a more indirect manner. For example phosphorylation of Snf3 by hexokinase PII could be followed by an inactivation or conformational change of Snf3. Since Snf3 is thought to be involved in the regulation of the hexose transporters, it is tempting to speculate that this would have its influence on the activity of the Hxt-proteins and therefore have an effect on the consumption rate. If important regulation on transport takes place on such a short time scale, discrepancies between in vivo and in vitro determined uptake rate are not so unlikely any more.

The results described here clearly demonstrate that performing kinetic analyses of sugar transport should be accompanied by a comparison with in vivo determined consumption rates. This paper clearly demonstrates that in vitro glucose and fructose uptake experiments do not give correct results, which means that the kinetic nature of such an uptake system cannot be determined under these conditions in contrast to kinetic analyses of galactose uptake.

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References

[1] Bisson, L.F. and Fraenkel, D.G. (1983) Proc. Natl. Acad. Sci. USA 80, 1730–1734.
[2] Bisson, L.F. and Fraenkel, D.G. (1984) J. Bacteriol. 159, 1013–1017.
[3] Lewis, D.A. and Bisson, L.F. (1991) Mol. Cell. Biol. 11, 3804–3813.
[4] Kruckeberg, A.L. and Bisson, L.F. (1990) Mol. Cell. Biol. 10, 5903–5913.
[5] Ko, C.H., Liang, H. and Gaber, R.F. (1993) Mol. Cell. Biol. 13, 638–648.
[6] Reifenberger, E., Freidel, K. and Ciriacy, M. (1995) Mol. Microbiol. 16, 157–167.
[7] Özcan, S. and Johnston, M. (1994) Mol. Cell. Biol. 15, 1564–1572.
[8] Sierkstra, L.N., Verbakel, J.M.A. and Verrips, C.T. (1992) J. Gen. Microbiol. 138, 2559–2566.
[9] Walsh, M.C., Smits, H.P., Scholte, M. and Van Dam, K. (1994) J. Bacteriol. 176, 953–958.
[10] Gimeno, C.J., Ljundahl, P.O., Styles, C.A. and Fink, G.R. (1992) Cell 68, 1077–1090.
[11] Ter Schure, E.G., Silljé, H.W., Raeven, L.J.R.M., Boonstra, J., Verkleij, A.J. and Verrips, C.Th. (1995) Microbiology 141, 1101–1108.
[12] Larsson, C., Von Stockar, U., Marison, I. and Gustafsson, L. (1993) J. Bacteriol. 175, 4809–4816.
[13] Rutgers, M., Balk, P.A. and Van Dam, K. (1990) Arch. Microbiol. 153, 478–484.
[14] Postma, E., Scheffers, W.A. and Van Dijken, J.P. (1989) Yeast 5, 159–165.
[15] Johnston, M. (1987) Microbiol. Rev. 51, 458–476.
[16] Wendell, D.L. and Bisson, L.F. (1993) J. Bacteriol. 175, 7689–7696.
[17] Vojtek, A.B. and Fraenkel D.G. (1990) Eur. J. Biochem. 190, 371–375.
[18] Kriegel, T.M., Rush, J., Vojtek, A.B., Clifton, D. and Fraenkel, D.G. (1994) Biochemistry 33, 148–152.
[19] Herrero, P., Fernández, R. and Moreno, F. (1989) J. Gen. Microbiol. 134, 2493–2498.
[20] Bisson, L.F., Coons, D.M., Kruckeberg, A.L. and Lewis, D.A. (1993) Crit. Rev. Biochem. Mol. Biol. 28, 259–308.