40 High affinity glucose transport in *Saccharomyces cerevisiae* deleted in hexokinase II

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Introduction

Glucose transport in *Saccharomyces cerevisiae* is mediated by members of the hexose transporter family (*HXT*'s) through facilitated diffusion. During growth on glucose the affinity of glucose transport changes (Walsh *et al.*, 1994), this coincides with a change in the hexose transporter genes expressed (Diderich *et al.*, 1999). At high glucose concentrations the kinetics of glucose transport are predominantly determined by low affinity transporters and when the glucose concentration decreases glucose transport is taken over by high affinity transporters. It was shown that the high affinity glucose transporter is subject to glucose repression (Entian and Frohlich, 1984; Bisson, 1988).

When glucose has entered the cell it is phosphorylated to glucose-6-phosphate. In the yeast *S. cerevisiae*, there are three enzymes that phosphorylate glucose: glucokinase, hexokinase I and hexokinase II (Lobo and Maitra, 1977). In previous studies it has been shown that hexokinase II is implicated in glucose repression (Entian and Frohlich, 1984; Gancedo, 1998). We are presently studying the effects of a hexokinase II deletion on the physiology of *S. cerevisiae*; here we will focus on the involvement of the hexose transport properties.

Materials and methods

Saccharomyces cerevisiae wild type strain CEN.PK 113-7D (*MAT***a**, *MAL2-8*^{c} *SUC2*) which was kindly provided by Dr. P. Kötter (Frankfurt, Germany) was used for the construction of a mutant strain which lacks the *HXK2* gene. The *HXK2* gene

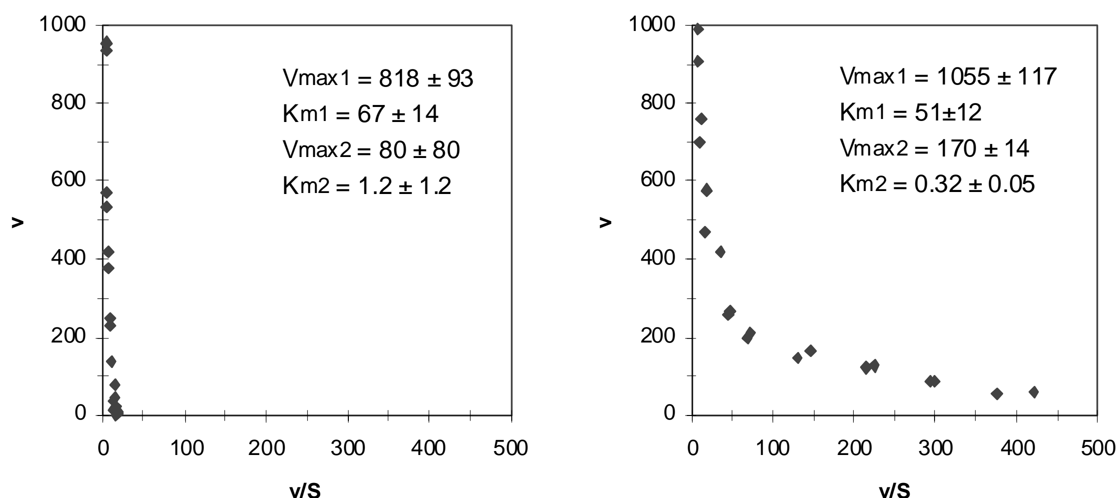

Fig. 40.1 Eadie-Hofstee plot of the kinetics of glucose transport. The wild type strain (left) and the hxk2 deficient mutant strain (right) were grown on YNB, 1% glucose. Zero-trans influx of ^{14}C-glucose was determined in cells harvested during exponential growth on glucose at an optical density at 600 nm of 0.5. Rate, v, is expressed in nmol/min/mg protein. Concentration of S is in mM.

was completely deleted by a PCR-based gene disruption and replaced by a kanMX-marker. Batch cultivations were performed in a bioreactor at 30°C at a stirring speed of 1000rpm. The growth medium contained 1% (w/v) glucose, 0.17% (w/v) Yeast Nitrogen Base without amino acids and ammonium sulphate (Difco), 0.5% (w/v) $(NH_4)_2SO_4$ and 0.1 M potassium phthalate at pH 5.0. Aerobic conditions were ascertained by aeration at 1 vessel volume of air per minute. During growth CO_2 evolution and oxygen consumption analysis was performed on-line with mass spectrometry.

In a parallel experiment yeast cells were grown to exponential phase (OD 600nm approximately 0.5, l = 1 cm, Pharmacia, Novaspec II), harvested by centrifugation at 5000 rpm (4°C), washed three times in ice-cold 0.1 M KH_2PO_4 buffer (pH 6.5) and resuspended in 0.1 M KH_2PO_4 buffer (pH 6.5). Zero-*trans* influx of ^{14}C-glucose was measured according to Walsh *et al.* (1994). Kinetic parameters of glucose transport were derived using Enzfitter software with proportional weighting.

Results and discussion

In a strain where hexokinase II is deleted there is a strong presence of high affinity glucose transport during exponential growth on glucose (Fig. 40.1). The strong presence of high affinity glucose transport in the mutant cells seems to

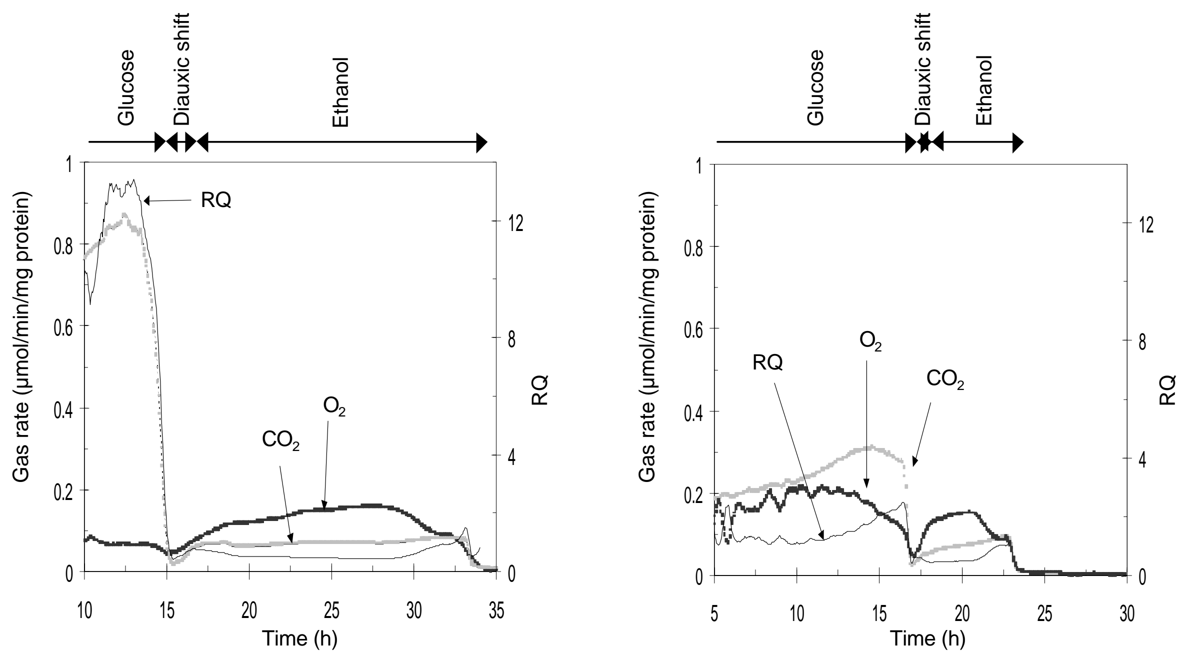

Fig. 40.2 Gas rates and Respiratory Quotient (RQ) during growth on glucose. The wild type strain (left) and the hxk2 deficient mutant strain (right) were grown on YNB, 1% glucose. Oxygen consumption and carbon dioxide production were measured online by a Mass Spectrometer. The RQ was determined by dividing the specific carbon dioxide production through the specific oxygen consumption. Growth phases are indicated above graphs.

be correlated with derepression of specific members of the *HXT*-family (Petit, T., manuscript in preparation). The properties of the high affinity component in the mutant strain (See Fig. 40.2) can not be explained by the separate affinities of the functional glucose transport proteins as determined previously (Reifenberger *et al.*, 1997). None of the separate hexose transport proteins shows an affinity for glucose that is as extremely high as displayed for the exponentially growing hexokinase II mutant cells. This points to an induction of a hexose transporter(s) that is non-functional in the wild-type cells or to the modulation of the glucose transport affinity (by phosphorylation, interaction with other proteins or dimerization).

The deletion of the *HXK2* gene has a remarkable effect on the physiological properties of the yeast. Growth on glucose is fermentative in wild type yeast under aerobic conditions. A large fraction of the glucose is converted into ethanol, a phenomenon known as the Crabtree-effect. In Fig. 40.2 this can be observed by the higher CO_2 evolution in comparison to the O_2 consumption (RQ$>$ 1). In hexokinase II deletants, growth is completely oxidative at high concentrations of glucose (Fig. 40.1). Glucose is not fermented but respired, which leads to an oxygen consumption equal to the carbon dioxide production (Respiratory Quotient $\approx$1). This results in higher biomass yields and lower ethanol production, which are interesting properties for industry.

Steady-state metabolite levels are determined by the kinetic characteristics of the metabolic machinery. The lower hexose-phosphorylating capacity and the presence of glucose transport with a very high affinity in the hexokinase II mutant during growth at a high glucose concentration might change the concentration of intracellular glucose or glucose-6-phosphate. Both intracellular glucose and glucose-6-phosphate have been suggested as a signal molecule for glucose repression (Gancedo, 1998).

Our goal now is to better understand the consequences of the hexokinase II deletion. Can we understand the changed phenotype by a survey of the physiological properties of the hexokinase II deletant? Is the hexokinase II deletant phenotype strictly a consequence of the change in affinity for glucose? Do other properties of the metabolic machinery change (metabolites, enzyme activities or enzyme affinities)? And can answers on these questions be obtained via a thorough quantitative experimental analysis coupled to metabolic modelling?

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