Volume 269, number 1, 202-204 FEBS 08763 August 1990

2-Deoxy-D-glucose resistant yeast with altered sugar transport activity

Srdjan Novak*, Tony D'Amore and Graham G. Stewart

Brewing Research Department, John Labatt Limited, 150 Simcoe Street, London, Ont., Canada N6A 4M3

Received 21 June 1990

The transport of glucose and maltose in *Saccharomyces cerevisiae* was observed to occur by both high and low affinity transport systems. A spontaneously isolated 2-deoxy-D-glucose resistant mutant was observed to transport glucose and maltose only by the high affinity transport systems. Associated with this was an increase in the V_{max} values, indicating derepression of the high affinity transport systems. The low affinity transport systems could not be detected. This mutant will be important in examining the repression regulatory and sugar transport mechanisms in yeast.

Sugar transport; 2-Deoxy-D-glucose; Glucose; Maltose; Yeast

1. INTRODUCTION

Kinetic studies have distinguished two transport systems for glucose in yeast; high and low affinity systems with K_m values of 2 mM and 20 mM, respectively [1]. The high affinity glucose transport system requires a functional *SNF3* gene and at least one of the cognate kinases for operation [1,2]. On the other hand, the low affinity glucose transport system is a constitutive, kinase-independent, facilitated diffusion process [3]. Transport of maltose into *Saccharomyces cerevisiae* is regarded as proton symport [4,5], consisting of two transport systems with K_m values of 4 mM and 70 mM for the high and low affinity systems, respectively [6]. Both components are rapidly inactivated by the catabolite repression mechanism upon switching from maltose to glucose medium [6,7].

Glucose or catabolite repression is a major regulatory mechanism in yeast. The non-metabolizable and toxic glucose analogue 2-deoxy-D-glucose (2-DOG) was employed to isolate a number of resistant mutants [8,9]. One particular mutant isolated from a haploid *Saccharomyces cerevisiae* strain exhibited alterations in glucose and maltose uptake [9]. This manuscript reports on the kinetic analysis of glucose and maltose transport in this 2-DOG resistant mutant and its parental strain.

2. MATERIALS AND METHODS

2.1. *Chemicals*

D-[U-^{14}C]Glucose (270 mCi/mmol) and D-[U-^{14}C]maltose (440 mCi/mmol) were obtained from ICN Biomedicals, Inc. (Irvine, CA).

Correspondence address: T. D'Amore, Brewing Research Department, John Labatt Limited, 150 Simcoe Street, London, Ont., Canada N6A 4M3

**Present address:* Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Yugoslavia

All other chemicals were obtained from commercial sources and were of the highest available purity.

2.2. *Yeast strains and growth medium*

The yeast strains employed in this study were, with the Labatt Culture Collection numbers, *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant strain 1620. The 2-DOG resistant mutant was isolated as described previously [8]. The yeast cells were subcultured in PYN medium as described previously [10].

2.3. *Fermentation conditions*

Fermentations were carried out at 21°C in 300 ml Erlenmeyer flasks containing 100 ml of media and shaken at 150 rpm on an orbital shaker. The yeast inoculum employed in all cases was 3.5 g wet weight cells/l. At specified times during fermentation, 10 ml of cell suspension was withdrawn. The samples were centrifuged at $4000 \times g$ for 10 min at 4°C. The supernatants were subjected to HPLC analysis for determining sugar concentrations as described previously [10].

2.4. *Glucose and maltose transport studies*

For glucose transport, glucose grown cells were washed twice with ice-cold 100 mM potassium phosphate buffer pH 6.6 and suspended in the same buffer at room temperature [10]. For maltose transport, maltose grown cells were washed twice with ice-cold distilled water and suspended in 100 mM tartaric acid-tris buffer, pH 4.2 [5]. The cell density employed in these studies was 30 mg wet weight/ml. Transport studies were initiated by addition of 1 ml of cell suspension to 2 ml of radioactive substrate to the desired final concentration as described previously [10]. Kinetic studies for glucose and maltose transport were conducted in the concentration range of 0.5–100 mM.

3. RESULTS

3.1. *Repression-derepression studies*

When the yeasts were inoculated into media containing a mixture of glucose and maltose (20 g/l of each sugar), the parental *Saccharomyces cerevisiae* strain 1190 clearly demonstrated maltose repression (Fig. 1A). That is, maltose uptake did not occur until most of the glucose was taken up from the medium. On the other hand, the uptake of maltose was observed to occur immediately in the 2-DOG resistant mutant, indicating an alteration in the glucose repression mech-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50 © 1990 Federation of European Biochemical Societies

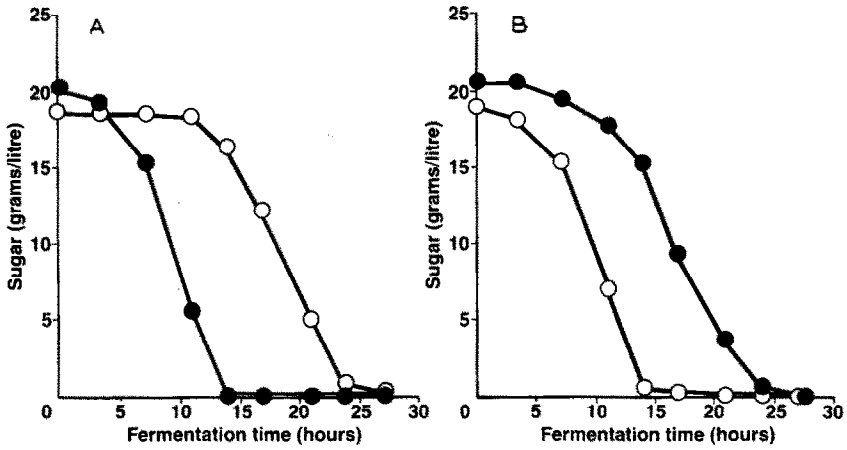

Fig. 1. Sugar uptake during fermentation of a 20 g/l glucose/20 g/l maltose medium. The strains employed were: (A) *Saccharomyces cerevisiae* 1190 and (B) its 2-DOG resistant mutant 1620. Glucose uptake (●) and maltose uptake (○).

anism (Fig. 1B). Furthermore, maltose was taken up approximately 2 times faster and glucose taken up approximately 2 times slower in the mutant strain compared to the parental strain.

3.2. *Glucose transport*

The results of the glucose transport studies in *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant is illustrated in Fig. 2. Glucose transport in the parental strain was observed to occur by two systems; high and low affinity transport systems with K_m values of 1.6 mM and 21.2 mM, respectively. The 2-DOG resistant mutant was observed to have only one transport system with a K_m value of 1.3 mM, representing the high affinity transport system. Furthermore, the V_{max} value was approximately 3 times higher in the 2-DOG resistant mutant suggesting that the high affinity glucose transport system is derepressed in the mutant. At the concentration range employed in this investigation, the low affinity glucose transport system was not detected in the mutant.

3.3. *Maltose transport*

Maltose transport in the parental strain was also observed to occur by two transport systems (Fig. 3). The K_m values were determined to be 1.6 mM and 40.4 mM for the high and low affinity transport systems, respectively. In the 2-DOG resistant mutant strain, only the high affinity transport system was detected, with a K_m value of 1.2 mM. The low affinity transport system could not be detected in the mutant within the concentration range employed. As in the case with glucose transport in the mutant, the V_{max} value was approximately 2 times higher for maltose transport in the mu-

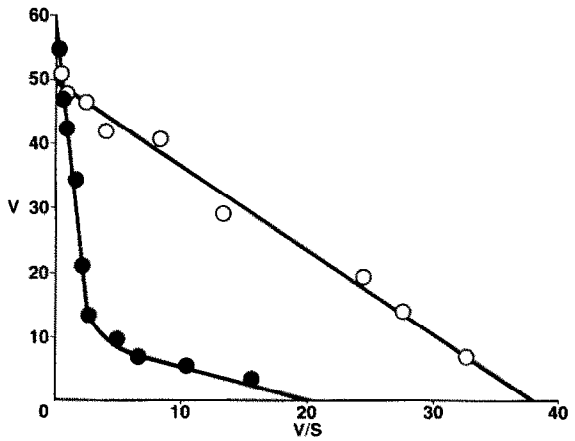

Fig. 2. Eadie-Hofstee plot of glucose transport. The strains employed were *Saccharomyces cerevisiae* 1190 (●) and its 2-DOG resistant mutant 1620 (○). The cells were grown on 40 g/l glucose to late-exponential phase. V, nmol/mg dry wt/min; S, glucose concentration, mM.

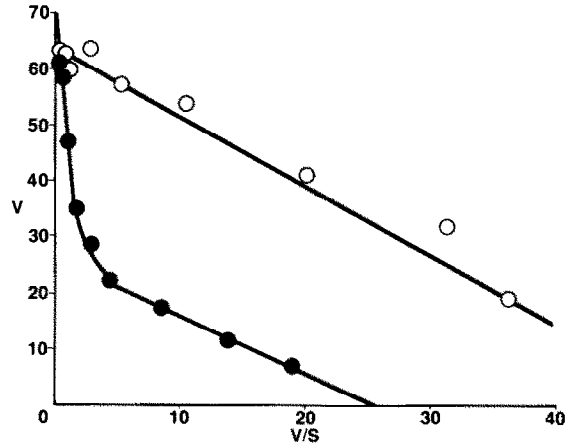

Fig. 3. Eadie-Hofstee plot of maltose transport. The strains employed were *Saccharomyces cerevisiae* 1190 (●) and its 2-DOG resistant mutant 1620 (○). The cells were grown on 40 g/l maltose to late-exponential phase. V, nmol/mg dry wt/min; S, maltose concentration, mM.

tant compared to the parental strain. This would suggest that the high affinity maltose transport system is further derepressed in the mutant.

4. DISCUSSION

Fermentation studies carried out on mixtures of glucose and maltose (Fig. 1A) confirmed that maltose uptake is strongly repressed in the parental strain as long as glucose is present in the medium [8,10]. On the other hand, maltose uptake in the 2-DOG resistant mutant was derepressed or insensitive to glucose repression (Fig. 1B). In previous studies, this mutant was also observed to be derepressed for sucrose and galactose uptake [9]. Furthermore, it was observed that maltose uptake was faster and glucose uptake slower in the mutant strain compared to the parental strain, suggesting alterations in glucose and maltose uptake [9]. Therefore, the kinetics of glucose and maltose transport in the parental and 2-DOG resistant mutant strains were investigated.

Analysis of glucose and maltose transport in the parental *Saccharomyces cerevisiae* 1190 strain revealed that both sugars were transported by biphasic kinetics, indicating the presence of high and low affinity transport systems (Figs 2 and 3). These results are in agreement with those reported in other studies [1,6,11]. The transport of glucose into the 2-DOG resistant mutant was observed to occur by the high affinity transport system. Associated with this was complete inactivation of the low affinity transport system. Furthermore, the V_{max} value was approximately 3 times higher in the mutant suggesting derepression of the high affinity transport system. The high affinity glucose transport system in *Saccharomyces cerevisiae* has been shown to be under general glucose repression control [1,12,14]. That is, the high affinity transport system was repressed when cells were grown in the presence of glucose. Cells which are fully repressed in the high affinity transport system exhibit only low affinity glucose transport. Likewise, cells which are derepressed in the high affinity transport system do not exhibit low affinity glucose transport [12,14]. Recent studies with 2-DOG resistant mutants of *Neurospora crassa* revealed a class of mutants in which glucoamylase, invertase and the high affinity glucose transport system were derepressed [15].

Maltose transport into the 2-DOG resistant mutant was also observed to occur only by the high affinity system. The V_{max} value was approximately 2 times higher in the mutant, suggesting that the high affinity maltose transport system was further derepressed. The low affinity transport system for maltose was also not detected in the mutant strain. Inactivation of the low affinity component of maltose transport in a glucose derepressed mutant has until now not been reported. This mutant will be important in examining the repression regulatory and sugar transport mechanisms in yeast.

Acknowledgements: The authors would like to thank Dr. I. Russell for helpful discussions in the preparation of this manuscript. The authors would also like to thank the management of John Labatt Limited for their support and permission to publish this manuscript.

REFERENCES

[1] Bisson, L.F. and Fraenkel, D.G. (1983) Proc. Natl. Acad. Sci. USA 80, 1730–1734.
[2] Bisson, L.F., Neigeborn, L., Carlson, M. and Fraenkel, D.G. (1987) J. Bacteriol. 169, 1656–1662.
[3] Lang, J.M. and Cirillo, V.P. (1987) J. Bacteriol. 169, 2932–2937.
[4] Seaston, A., Inkson, C. and Eddy, A.A. (1973) Biochem. J. 134, 1031–1043.
[5] Serrano, R. (1977) Eur. J. Biochem. 80, 97–102.
[6] Busturia, A. and Lagunas, R. (1985) Biochim. Biophys. Acta 820, 324–326.
[7] Holzer, H. (1976) Trends Biochem. Sci. 1, 178–180.
[8] Jones, R.M., Russell, I. and Stewart, G.G. (1986) J. Am. Soc. Brew. Chem. 44, 161–166.
[9] Novak, S., D'Amore, T., Russell, I. and Stewart, G.G. (1990) J. Ind. Microbiol. (in press).
[10] D'Amore, T., Russell, I. and Stewart, G.G. (1989) J. Ind. Microbiol. 4, 315–324.
[11] Ramos, J., Szkutnicka, K. and Cirillo, V.P. (1988) J. Bacteriol. 170, 5375–5377.
[12] Bisson, L.F. and Fraenkel, D.G. (1984) J. Bacteriol. 159, 1013–1017.
[13] Bisson, L.F. (1988) J. Bacteriol. 170, 2654–2658.
[14] Bisson, L.F. (1988) J. Bacteriol. 170, 4838–4845.
[15] Allen, K.E., McNally, M.T., Lowerdorf, H.S., Slayman, C.W. and Free, S.J. (1989) J. Bacteriol. 171, 53–58.