SHORT COMMUNICATION

Changes in Glucose 6-Phosphate and Storage Carbohydrates during Catabolite Derepression in *Saccharomyces cerevisiae*

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Changes in the cellular content of glucose 6-phosphate and storage carbohydrates during catabolite derepression were measured during the diauxic growth of *Saccharomyces cerevisiae* on glucose (1 %, w/v) medium. After the exhaustion of glucose there were transient increases in glucose 6-phosphate, alkali-soluble glycogen and trehalose, reflecting enhanced gluconeogenesis, followed by an increase in acid-soluble glycogen. During the subsequent adaptation to growth on ethanol the amounts of glucose 6-phosphate, trehalose and glycogen returned to basal levels. Thus storage carbohydrates, of which glycogen is quantitatively the most important, appear to act as the energy source for catabolite derepression.

INTRODUCTION

The polysaccharides of *Saccharomyces cerevisiae* are chemically heterogeneous. They can be classified and fractionated into two main groups: (i) structural wall-located carbohydrates, which include a β -glucan and a mannan, and (ii) storage carbohydrates consisting of glycogen and the disaccharide trehalose (Manners, 1971).

Catabolite derepression characterized by the transition from fermentative to oxidative growth (for review, see Linnane & Haslam, 1970) results in an accumulation of trehalose (Panek, 1962; Panek & Mattoon, 1977) and a glycogen (Polakis & Bartley, 1966). During subsequent oxidative growth these reserves are utilized as a source of energy for cell growth (Panek, 1963; Küenzi & Fiechter, 1969).

Panek & Mattoon (1977) have suggested that trehalose degradation may be the chief source of energy which drives mitochondrial biogenesis. However, some yeast strains accumulate trehalose after the beginning of ethanol oxidation, which would argue against such a role (Costa-Carvalho *et al.*, 1978).

The object of this investigation was to monitor in greater detail the changes in reserve and structural carbohydrates during catabolite derepression.

METHODS

Growth media and the yeast strain, AB1, were as described previously (Quain & Haslam, 1979). Yeast (10 mg dry wt) was inoculated into 1 l glucose (1 %, w/v) medium and grown aerobically at 30 °C on a rotary shaker (180 rev. min⁻¹) for 22 h. Samples were taken for glucose analysis, dry weight determination and carbohydrate fractionation at intervals between 10 and 22 h. The experiments were done in duplicate. Yeast samples (40 mg dry wt) were washed three times with ice-cold water and stored at -17 °C prior to analysis. Carbohydrates were fractionated according to the method of Trevelyan & Harrison (1956) as reported by Stewart (1975), and fractions were analysed in triplicate using the anthrone procedure (Stewart, 1975). Glucose and dry weight were determined as described previously (Quain & Haslam, 1979).

Changes in intracellular glucose 6-phosphate concentration were investigated by removing samples (10 mg dry wt) at intervals of 15 min from a culture undergoing catabolite derepression. To facilitate rapid sampling with the minimum of metabolic perturbation, a modular fermentation system was used (Quain & Haslam, 1979) with an agitation rate of 300 rev. min⁻¹ and aeration at 0.33 culture vol. min⁻¹. Cells were harvested by vacuum filtration, and the cellulose acetate filter was transferred to a beaker with 1.5 ml perchloric acid (5 %, v/v) at 0 °C. The filter was removed and washed with 0.5 ml perchloric acid. The cell suspension was freeze-thawed three times and then centrifuged at 40000 *g* for 5 min at 2 °C. Acid-washed charcoal (20 mg) was added to the supernatant which was then neutralized dropwise with potassium hydroxide (80 %, w/v) and maintained at 0 °C for 1 h. Precipitated potassium perchlorate was removed by centrifuging at 40000 *g* for 20 min, and residual charcoal was removed by filtration through acid-washed glass-fibre paper. Samples were stored at -70 °C prior to assay. Glucose 6-phosphate was assayed in duplicate using the fluorometric procedures of Lowry & Passonneu (1972) in the concentration range 0.05 to 0.2 μ M.

RESULTS AND DISCUSSION

Cells growing on medium containing glucose (1 %, w/v) exhibit diauxic behaviour with respect to growth and physiology (for review, see Linnane & Haslam, 1970). This provides a convenient system for the investigation of the transition between catabolite repressed and catabolite derepressed states. During the initial exponential growth phase on glucose medium, aerobic glycolysis is responsible for the production of cellular energy and the provision of carbon skeletons for biosynthesis. After exhaustion of the glucose, a 3 h adaption period is observed during which there is no cellular growth. This transition period marks the onset of catabolite derepression. After the adaption period, mitochondria become fully functional and enable oxidative growth at a slower but exponential rate using the ethanol in the medium as source of carbon skeletons and energy.

Changes in structural and reserve carbohydrates during diauxic growth are shown in Fig. 1. The total carbohydrate of the cells doubled during growth on glucose, reached a maximum 1 h after glucose exhaustion, and then declined. Alkali-soluble glycogen also exhibited a peak 1 h after glucose exhaustion but returned in the next hour to a basal level. Acid-soluble glycogen reached a peak 3 h after the onset of the adaptive phase and then fell to a new level, somewhat lower than that during fermentative growth. The precise nature, function and interrelationship of the two types of glycogen is not known, but it is reasonable to suggest that the disappearance of the alkali-soluble glycogen peak and the appearance of a peak of acid-soluble glycogen, comparable in amount, represents an interconversion of the two forms. However, 2 h after the exhaustion of glucose, the decrease in alkali-soluble glycogen was not immediately accompanied by an increase in acid-soluble glycogen. This is not explicable if there is a direct precursor relationship between the two forms of glycogen, but may arise through a technical difficulty in the extraction of an intermediate form of the polymer, which would also explain the apparent decrease in total carbohydrate at this time. The changes in trehalose concentration were slower and smaller in amount, being characterized by a gradual accumulation to a maximum value 1 h after glucose exhaustion, followed by a slow decline reaching a basal level 3 h later.

The non-proliferating conditions found during the adaptive phase are optimum for trehalose synthesis (Panek, 1962) and accumulation of trehalose has previously been reported either during (Costa-Carvalho *et al.*, 1978) or before adaption to growth on ethanol (Panek & Mattoon, 1977). The slow utilization of trehalose may reflect an increase in the activity of trehalase in response to an increase in intracellular cyclic AMP concentration (van der Plaats & van Solingen, 1974) which occurs during catabolite derepression (van Wijk & Konijn, 1971; Mahler & Lin, 1978). Trehalose hydrolysis during this period may be of importance in providing energy during the continuation of mitochondrial development, but it is unlikely to be the sole source of energy for the initiation of biogenesis as suggested by Panek & Mattoon (1977). The present results show that glycogen is also probably involved, and indeed appears to be quantitatively more important.

Fig. 1

Fig. 2

Fig. 1. Changes in reserve and structural carbohydrates during diauxic growth on glucose medium. Cells were grown and carbohydrates were fractionated as described in Methods. Glucose was exhausted at 0 h. Concentrations of acid-soluble glycogen (■), alkali-soluble glycogen (○), trehalose (▲), mannan (△), total carbohydrate (●) and cell dry weight (□) are indicated. All points represent the arithmetical mean of triplicate determinations of each component in two separate experiments. The variation of each point was less than 2% except for three of the points indicating total carbohydrate where the bars indicate the ranges.

Fig. 2. Changes in intracellular glucose 6-phosphate, medium glucose concentration and cell dry weight before and during the catabolite derepression transition. Glucose 6-phosphate (□), cell dry weight (●) and glucose (○) were analysed as described in Methods. Glucose was exhausted at 0 h.

The biosynthesis of trehalose requires uridine 5'-diphosphate glucose (UDPG) and glucose 6-phosphate whereas glycogen synthesis requires UDPG and a polysaccharide primer (Manners, 1971). Hence, as glucose 6-phosphate is involved in the synthesis of UDPG, it would appear that this compound may play a pivotal role in any hypothesis which explains the observed changes in reserve carbohydrate.

Figure 2 shows the intracellular glucose 6-phosphate concentration before and during catabolite derepression. The concentration of this metabolite reached a maximum 2 h before glucose exhaustion when the glucose concentration had been depleted to 0.5% (w/v) (initial concentration 1%, w/v); glucose 6-phosphate concentration then rapidly declined concomitantly with the utilization of the remaining glucose. However, 45 min after glucose exhaustion, there was a transient increase in glucose 6-phosphate but this rapidly returned to a basal level.

These results may be interpreted as indicating that when the cells stop growing due to the glucose exhaustion the available energy (probably mainly ATP, but possibly also polyphosphate) is used to drive the formation of glucose 6-phosphate from ethanol by gluconeogenesis. Enzymes unique to gluconeogenesis are phosphoenolpyruvate carboxykinase (EC 4.1.1.31), pyruvate carboxylase (EC 6.4.1.1) and fructose-1,6-bisphosphatase (EC 3.1.3.11), which have all been shown to increase during catabolite derepression in yeast (Haarasilta & Oura, 1975). Further, Barwell & Hess (1971) have observed a steady

rate of polysaccharide synthesis as a result of gluconeogenesis, and Maitra & Lobo (1978), in a detailed study of glycolytic reversal, showed a rapid flux from phosphoenolpyruvate to hexose monophosphates when ethanol was added to derepressed cells.

The glucose 6-phosphate formed by gluconeogenesis may then be utilized in the synthesis of trehalose and alkali-soluble glycogen, the latter possibly being converted to the acid-soluble form within 2 h. The utilization of reserve carbohydrates, both trehalose and glycogen, during the adaptive phase of catabolite derepression may provide energy for the synthesis of mitochondrial and other enzymes needed for growth on ethanol.

Finally, structural carbohydrate (mannan, see Fig. 1, and glucan, not shown) showed a considerable increase during fermentative growth reaching a maximum at the time of glucose exhaustion. The new level was maintained throughout catabolite derepression.

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REFERENCES

- BARWELL, C. J. & HESS, B. (1971). Regulation of pyruvate kinase during gluconeogenesis in *Saccharomyces cerevisiae*. *FEBS Letters* **19**, 1-4.
- COSTA-CARVALHO, V. L. A., PANEK, A. & MATTOON, J. R. (1978). Effects of carbon source and genetic modifications of phosphoglucomutase in trehalose metabolism in *Saccharomyces cerevisiae*. *FEMS Microbiology Letters* **4**, 221-224.
- HAARASILTA, S. & OURA, E. (1975). On the activity and regulation of anaplerotic and gluconeogenic enzymes during the growth process of bakers' yeast. The biphasic growth. *European Journal of Biochemistry* **52**, 1-7.
- KÜENZI, M. T. & FIECHTER, A. (1969). Changes in carbohydrate composition and trehalase activity during the budding cycle of *Saccharomyces cerevisiae*. *Archiv für Mikrobiologie* **64**, 369-407.
- LINNANE, A. W. & HASLAM, J. M. (1970). The biogenesis of yeast mitochondria. *Current Topics in Cellular Regulation* **2**, 101-172.
- LOWRY, O. H. & PASSONNEU, J. V. (1972). *A Flexible System of Enzymatic Analysis*. London & New York: Academic Press.
- MAHLER, H. R. & LIN, C. C. (1978). Exogenous adenosine 3':5'-monophosphate can release yeast from catabolite repression. *Biochemical and Biophysical Research Communications* **83**, 1039-1047.
- MAITRA, P. K. & LOBO, Z. (1978). Reversal of glycolysis in yeast. *Archives of Biochemistry and Biophysics* **185**, 535-543.
- MANNERS, D. J. (1971). The structure and biosynthesis of storage carbohydrates in yeast. In *The Yeasts*, 1st edn, vol. 2, pp. 419-439. Edited by A. H. Rose & J. S. Harrison. London & New York: Academic Press.
- PANEK, A. (1962). Synthesis of trehalose by bakers yeast (*Saccharomyces cerevisiae*). *Archives of Biochemistry and Biophysics* **98**, 349-355.
- PANEK, A. (1963). Function of trehalose in bakers yeast (*Saccharomyces cerevisiae*). *Archives of Biochemistry and Biophysics* **100**, 422-425.
- PANEK, A. & MATTOON, J. R. (1977). Regulation of energy metabolism in *Saccharomyces cerevisiae*. Relationship between catabolite repression, trehalose synthesis and mitochondrial development. *Archives of Biochemistry and Biophysics* **183**, 306-316.
- PLAAT, J. B. VAN DER & SOLINGEN, P. VAN (1974). Cyclic 3',5'-adenosine monophosphate stimulates trehalose degradation in bakers yeast. *Biochemical and Biophysical Research Communications* **56**, 580-587.
- POLAKIS, E. S. & BARTLEY, W. (1966). Changes in dry weight, protein, deoxyribonucleic acid, ribonucleic acid and reserve and structural carbohydrates during the aerobic growth cycle of yeast. *Biochemical Journal* **98**, 883-887.
- QUAIN, D. E. & HASLAM, J. M. (1979). The effects of catabolite derepression on the accumulation of sterol esters and activity of β -hydroxymethylglutaryl-CoA reductase in *Saccharomyces cerevisiae*. *Journal of General Microbiology* **111**, 343-351.
- STEWART, P. R. (1975). Analytical methods for yeasts. In *Methods in Cell Biology*, vol. 12, pp. 111-147. Edited by D. M. Prescott. New York: Academic Press.
- TREVELYAN, W. E. & HARRISON, J. S. (1956). Studies on yeast metabolism. 7. Yeast carbohydrate fractions. Separation from nucleic acid, analysis and behaviour during anaerobic fermentation. *Biochemical Journal* **63**, 23-33.
- WIJK, R. VAN & KONIJN, T. M. (1971). Cyclic 3',5'-AMP in *Saccharomyces carlsbergensis* under various conditions of catabolite repression. *FEBS Letters* **13**, 184-186.