

Changes in fermentative capacity during nitrogen starvation in baker's yeast are initially regulated by multi-site modulation

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Introduction

The most important characteristic of baker's yeast (*Saccharomyces cerevisiae*) is its capacity to ferment sugars to ethanol and carbon dioxide. The yeast production process is terminated by starving the cells for nitrogen in order to increase the carbohydrate content, which is believed to increase the storage stability (Caron, 1995; Reed and Nagodawithana, 1991). Nitrogen starvation leads to a (partial) loss of the fermentative capacity. The growth conditions and the obtained fermentative capacity prior to the nitrogen starvation is an important parameter for the magnitude of this loss in fermentative capacity. Results of several studies suggest that a high initial fermentative capacity is more instable during nitrogen starvation (Rossell *et al.*, 2007; Rossell *et al.*, 2006). To get a better insight in this problem we studied the regulation of fermentative capacity as a function of time.

Methods

Cells grown in aerobic glucose-limited chemostats at a specific growth rate of 0.35 h⁻¹ were subjected to nitrogen starvation conditions. Excess glucose was added to prevent double starvation. Samples were taken, up to 24 hours for measuring the fermentative capacity and the activities (V_{\max}) of the glycolytic and fermentative enzymes. The obtained data were used to determine the hierarchical regulation coefficients using time-dependent regulation analysis (Bruggeman *et al.*, 2006), by the following equation:

$$\rho_h(t) = \frac{\ln V_{\max}(t) - \ln V_{\max}(t_0)}{\ln v(t) - \ln v(t_0)} \quad [1]$$

Regulation analysis allows to dissect the regulation of the flux through an individual enzyme in a hierarchical or gene-expression component (through changes in maximum enzyme activity V_{\max}), and a metabolic component (through changes in the interaction of the enzyme with the rest of metabolism). According to the summation theorem for the regulation of the flux

the hierarchical regulation coefficient (ρ_h) and metabolic regulation coefficient (ρ_m) sum up to 1 (Bruggeman *et al.*, 2006; Rossell *et al.*, 2005; ter Kuile and Westerhoff, 2001):

$$1 = \rho_h(t) + \rho_m(t) \quad [2]$$

Although the regulation coefficients concern local flux regulation, they also give insight in global flux regulation. We can distinguish various paradigms of global regulation, (i) modulation of single rate-limiting enzymes, (ii) multi-site modulation, *i.e.* simultaneous and proportional modulation of all the enzymes in the pathway (Fell and Thomas, 1995), (iii) exclusive metabolic regulation and (iv) enzymes play different regulatory roles (Rossell *et al.*, 2006).

Results and discussion

After 24 hours of nitrogen starvation the fermentative capacity was decreased by 35% and various combinations of metabolic and hierarchical regulation were observed. This suggested that enzymes played different roles in the regulation of the pathway's flux, as was observed earlier for batch-grown cells (Rossell *et al.*, 2006). However, during the first hours of nitrogen starvation a reduced fermentative capacity was accompanied by a reduced capacity (V_{\max}) of nearly all glycolytic and fermentative enzymes. The extent of the reduction in V_{\max} was such that most enzymes were regulated to a large extent hierarchically. This is consistent with a mechanism of unspecific 'bulk' degradation of proteins via autophagy in the first phase. Only phosphoglucose isomerase and glyceraldehyde-3-phosphate dehydrogenase escaped this rapid breakdown, or their breakdown was compensated for by new synthesis. Altogether we conclude that the fermentative capacity is predominantly regulated by multi-site modulation of the enzymes in the initial phase of nitrogen starvation.

References

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