The Influence of Ammonium Permease Activity and Carbon Source on the Uptake of Ammonium from Simple Defined Media by *Saccharomyces cerevisiae*

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When growing under defined conditions, cells of the yeast *Saccharomyces cerevisiae* absorbed ammonium more rapidly with glucose as carbon source than with maltose. Ammonium pool sizes and permease activity were also higher in the glucose-grown cells and the relationship implies that firstly, the sugar is a primary determinant of ammonium permease activity and, secondly, the activity of the permease largely determines both the rate of ammonium uptake and ammonium pool size in the first part of the fermentation.

**INTRODUCTION**

The plasma membrane of yeast cells is permeable to free ammonia in solution but at the pH of most yeast fermentations the compound is present almost entirely as the ammonium ion and this form requires an active uptake process (Roon *et al.*, 1975). Ammonium permease has been shown to be separate from the amino acid permeases (Roon *et al.*, 1977a) and the mutually inhibitory effects found in both transport systems have been explained by assuming competition for a common energy source rather than through an affinity of the inhibiting molecule for the other transport system (Roon *et al.*, 1977b). In simple defined media, ammonium may be the only source of nitrogen for synthesis of amino acids, proteins and nucleic acids. It is used very efficiently by *Saccharomyces cerevisiae* and, as a sole source of nitrogen, is only bettered by asparagine, aspartic acid and glutamine (Thorne, 1945; Dubois *et al.*, 1974), but there are a number of reports that ammonium may have other significances as well. The studies of Yoshino & Murakami (1982) with permeabilized yeast showed that ammonium could activate phosphofructokinase and pyruvate kinase and they suggested a role for ammonium control of glycolysis. On the basis of fermentation studies, Saita & Slaughter (1984) felt that the action of ammonium on glycolysis was better understood as a secondary effect arising from stimulation of protein synthesis by ammonium in its capacity as a nitrogen source. However, in experiments with intact cells of *S. carlsbergensis*, Lloyd *et al.* (1983) showed that addition of ammonium resulted in a short-lived increase in CO₂ output over a 5–10 min period, which could well have been due to direct activation of the glycolytic enzymes by ammonium. Another unexpected effect of ammonium was noted by Slaughter & Housden (1978), who reported that high levels of ammonium in excess of that required for growth caused a decrease in the formation of higher alcohols but a slight increase in the production of ethanol. The mechanism of this effect remains unknown, but Devine & Slaughter (1980) found that it occurred only in the presence of glucose and not with a range of other fermentable sugars.

Assimilation of ammonium within the cell into glutamate and other amino acids has been well studied in yeast (Brown, 1980) but little is known about the control of ammonium uptake. This paper describes experiments carried out to assess the importance of the permease system in control of ammonium uptake by *S. cerevisiae* during fermentation of defined sugar/salts media.
METHODS

Yeast maintenance and propagation. Saccharomyces cerevisiae NCYC 1108 was maintained on malt agar slopes at 4 °C. As required, yeast was pre-cultured in a malt extract medium for 3 d at 25 °C, harvested by centrifugation, washed twice with chilled distilled water and finally resuspended in chilled water at approximately 3 × 10⁹ cells ml⁻¹ prior to inoculation into the experimental media.

Experimental fermentation. The composition of the defined medium was described by Harding et al. (1984). All media contained ammonium sulphate at 1.18 g l⁻¹ and either glucose at 80 g l⁻¹ or maltose at 76 g l⁻¹. Fermentations were carried out at 20 °C in 500 ml conical flasks containing 250 ml medium and sealed with a fermentation lock. The flasks were incubated either without disturbance or on an orbital shaker at 130 r.p.m. The initial cell count was approximately 6 × 10⁶ ml⁻¹ as determined using the improved Neubauer haemocytometer.

In all the experiments described in this paper fermentations were carried out in duplicate and sampling for each determination was also in duplicate. The results shown are the means.

Determination of sugar and ammonium concentration. The concentrations of glucose and maltose were determined in samples from which the cells had been removed by centrifugation, using the reducing sugar determination of Somogyi & Nelson (1944). The ammonium concentration of the samples was determined using the urea/ammonia kit supplied by Boehringer Mannheim.

Determination of ammonium pool size. A sample of fermenting medium was passed through a Nuflow membrane filter (0.45 μm pore size) and the retained yeast washed twice with 10 ml portions of chilled water. The filter was then transferred to a tube containing boiling water and boiling was continued for a further 20 min. The aqueous extract was then cooled, clarified by centrifugation and made up to the original sample volume with distilled water before assay of the ammonium content using the technique described in the preceding section. Preliminary experiments showed that if the cells were washed only once then the results were variable. Two washes gave consistent results and the values were unaffected by a third wash.

Determination of ammonium permease activity. The [¹⁴C]methyl ammonium analogue assay described by Roon et al. (1975) was used with 10 mM-sodium citrate buffer, pH 6.5, and a cell density of 2.61 × 10⁷ cells ml⁻¹. The sugar used in the assay was the same as that present in the fermentation medium.

RESULTS

Influence of culture conditions on the uptake of sugar and ammonium. The fermentation of the defined medium containing either glucose or maltose as carbon source was followed under non-shaken and shaken conditions. Whereas there was a greater increase in cell number in the shaken cultures, the rate of uptake of ammonium from a particular medium was more or less unaffected (Fig. 1). In contrast, shaking the flasks caused a slight reduction in the rate of uptake of maltose and a distinct reduction in the rate of glucose uptake. Under both shaken and non-shaken conditions the rate of uptake of ammonium was greater in the presence of glucose as compared to maltose.

Influence of carbon source on the intracellular ammonium concentration. Measurement of ammonium pool sizes during fermentation under shaken conditions (Fig. 2a) showed a distinct peak at 5–10 h in the presence of either glucose or maltose but the pool size in the presence of glucose was persistently higher than in the presence of maltose.

Influence of carbon source on the ammonium permease activity of the cells. As was the case for the ammonium pools, ammonium permease showed a peak during fermentation of either glucose or maltose in shaken cultures (Fig. 2). The peak in permease activity appeared to occur just after the peak in pool size and once again, the activity in the presence of glucose was consistently higher than in the presence of maltose.

The uptake activities found ranged between 17.3 nmol methylamine ml⁻¹ min⁻¹ for cells from the glucose medium after 10 h and 2.9 nmol ml⁻¹ min⁻¹ for cells from the maltose medium after 24 h. The variation between duplicates was normally less than 5% and in no case greater than 10%.

DISCUSSION

Initial experiments (Fig. 1) showed that shaking appeared to allow a more efficient use of the sugars, particularly glucose, in terms of cell production. However, the initial rate of ammonium uptake was unaffected by shaking but was dependent on the carbon source under both the fermentation conditions tested, with a higher rate of uptake occurring in the presence of glucose.
NH₄⁺ permease control of NH₄⁺ uptake by yeast

Fig. 1. Effect of fermentation conditions on ammonium uptake (see Methods for experimental details). (a) Shaken culture. ○, Glucose concentration; □, maltose concentration (as hexose); ●, cell number in glucose medium; ■, cell number in maltose medium. (b) Shaken culture. ○, ammonium concentration in glucose medium; □, ammonium concentration in maltose medium. (c) Non-shaken culture. Symbols as in (a). (d) Non-shaken culture. Symbols as in (b).

Fig. 2. (a) Effect of sugar on the internal concentration of ammonium and ammonium uptake ability. See Methods for experimental details. ○, □. Concentration of ammonium in cells from glucose medium (○) and from maltose medium (□). ●, ■. Ammonium uptake ability of cells from glucose medium (●) and from maltose medium (■). (b) Effect of sugar on the relative changes in ammonium permease activity and internal ammonium pool during fermentation. ○, Ratio of the ammonium permease activity in cells from glucose as against maltose media; □, ratio of the ammonium pool size in cells from glucose as against maltose media.

This observation was reinforced by measurement of the ammonium pool size, which was also higher when glucose was being fermented as compared with maltose (Fig. 2a). As the amount of growth was not affected by the carbon source, although the uptake rate was, it seems unlikely that different rates of assimilation of ammonium into amino acids could account for differences in pool size. This supposition is supported by earlier measurements made on cells cultivated in the same media under non-shaken conditions (Saita, 1983), which showed that NADP-
dependent glutamate dehydrogenase activity was not affected by carbon source and, furthermore, was at an approximately tenfold excess as compared with the observed rate of ammonium uptake.

These data implied that the permease activity itself could be important in limiting uptake and in determining pool size. Fig. 2(a) shows that permease activity is dependent on the sugar and varies throughout fermentation. When the relative changes in permease activity in glucose and maltose media were compared with the relative changes in pool size under the same conditions (Fig. 2b), these two factors are seen to mirror each other up to 15 h, although the relative changes in pool size are not as great as those in permease activity. These results support the suggestion that ammonium permease activity is a major determinant of both the rate of ammonium uptake and pool size, certainly in the first part of the fermentation. Further weight is given to this view by a comparison between the observed rate of ammonium uptake during fermentation and that calculated from the cell number and the ammonium permease specific activity (Fig. 3). For the first 15 h, both sets of data have roughly similar values and show an upward trend in the case of both glucose and maltose. Given the errors and assumptions involved in a comparison of this type the agreement over the first 15 h seems quite good. The sharp fall-away in the calculated uptake rate and divergence from the observed rate after 15 h cannot be explained at the moment but it is clear that factors other than the ammonium permease activity must become progressively more important in determining both uptake rate and pool size as the fermentation proceeds.

It also appears that the sugar present in the medium affects the ammonium permease activity and so, indirectly, exerts an influence on the rate of ammonium uptake and pool size in the first part of the fermentation, but the mechanism of this phenomenon is unknown. The metabolism of glucose and maltose is so similar that it seems unlikely that a sugar metabolite is involved, but it is possible to speculate, as the two sugars are transported into the cell by different systems, that interaction with ammonium uptake could occur at this level. It could be that continual synthesis of the relatively unstable proteins involved in maltose transport and hydrolysis (Lagunas et al., 1982) consumes a significant quantity of the ATP generated within the cell and so reduces the amount of energy available to drive the active transport of compounds such as ammonium. Another potential mechanism which may be worthy of consideration is that for any given amount of hexose taken up, twice as many glucose molecules as compared to maltose molecules have to be transported and, if there were an element of cotransport between sugar and ammonium, this could provide an explanation for the effect of sugar on ammonium uptake.
NH₃⁺ permease control of NH₃⁺ uptake by yeast

REFERENCES


