SHORT COMMUNICATION

Correlation of Glutamine Synthetase Activity with Cell Magnesium Concentration during Cell Division in Yeast Synchronized by Induction

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Synchronization of cell division in the fission yeast Schizosaccharomyces pombe and the budding yeast Kluyveromyces fragilis was achieved by induction using the DNA synthesis inhibitor 2'-deoxyadenosine and by a magnesium-exhaustion technique. The activity of glutamine synthetase in these synchronized cultures oscillated. Variations in the intracellular magnesium concentration were also observed, and peaks in magnesium concentration correlated with peaks in enzyme activity. We suggest that the enzyme from yeast is unstable and that its activity is regulated in vivo by changes in the intracellular concentration of magnesium.

INTRODUCTION

Glutamine is central to many aspects of nitrogen metabolism as it serves as a nitrogen donor for many nitrogenous metabolites (Stadtman, 1973). Glutamine synthetase [L-glutamate : ammonia ligase (ADP-forming) EC 6.3.1.2] catalyses the ATP-driven synthesis of glutamine from glutamate and ammonia. Previous work in this laboratory (Duffus et al., 1974), and elsewhere (Holzer, 1969; Hachimori et al., 1974; Sims et al., 1974a, b), has indicated that the enzyme from a number of sources, including yeast, is regulated by complex allosteric mechanisms which involve magnesium. The aim of the present work was to investigate the effect of changes in the intracellular concentration of magnesium on glutamine synthetase activity during the cell cycle in yeast.

METHODS

Organisms and growth conditions. Schizosaccharomyces pombe NCYC 132 (ATCC 24751) and Kluyveromyces fragilis NCYC 100 were grown in Edinburgh Minimal Medium no. 2 (EMM2) (Mitchison, 1970) at 32 °C and 30 °C, respectively. Viable stock cultures were maintained by weekly subculture of stationary phase cells. Cell numbers were determined as described by Walker & Duffus (1980).

Synchronization techniques. 2'-Deoxyadenosine-pulse and magnesium-exhaustion synchronization of cell division was established in S. pombe and K. fragilis by the method of Walker & Duffus (1980).

Preparation of extracts and assay of glutamine synthetase. Cell-free extracts (crude enzyme preparation)

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Fig. 1. Glutamine synthetase activity and the intracellular concentration of magnesium in cultures of *S. pombe* (a) and *K. fragilis* (b) synchronized by pulsing with 2'-deoxyadenosine. Exponentially growing cells were treated with 2 mm 2'-deoxyadenosine for 5 h (*S. pombe*) or 2 h (*K. fragilis*), harvested, and resuspended into fresh EMM2 (at zero time).  
- , log<sub>10</sub> (cell number ml<sup>-1</sup>); ■, glutamine synthetase activity (ΔAA<sub>540</sub> min<sup>-1</sup> (10<sup>10</sup> cells)<sup>-1</sup>); △, intracellular magnesium concentration (f g cell<sup>-1</sup>).  

Control cultures (not shown), which lacked inhibitor treatment, grew asynchronously with doubling times of 150 min (*S. pombe*) and 100 min (*K. fragilis*). Cycle times for both synchronous cultures were extended by about 20 min following treatment with 2'-deoxyadenosine. Engleberg Synchronization Indices (Engleberg, 1961) for the first synchronous divisions were 44% (*S. pombe*) and 27% (*K. fragilis*), as calculated by the method of Bakke & Petersen (1976).

were prepared from 500 ml culture harvested by Millipore membrane (1-2 μm pore size) filtration. Cells were washed once on the filter with glass-distilled water, immediately frozen in an ethanol cold bath at −45 °C, and stored at −18 °C. After 1 d (since prolonged freezing reduced enzyme activity), the cells were thawed at room temperature, resuspended in 2 ml imidazole hydrochloride buffer (0.1 M, pH 7.1), and homogenized by a single passage through an Eaton press (Eaton, 1962) previously cooled to −45 °C (this ensured complete disruption of cells). The extract was finally thawed at room temperature and debris was removed by centrifuging at 3000 g for 20 min. In a modification of the method of Shapiro & Stadtman (1970), the supernatant was assayed immediately for glutamine amido-transferase activity in a freshly prepared mixture containing (final concentrations) glutamine (0.1 M), MnCl<sub>2</sub> (0.1 M), ADP (0.01 M, pH 6.5), potassium arsenate (1.0 M, pH 6.5) and hydroxylamine (2.0 M), all in 0.1 M-imidazole hydrochloride buffer (pH 6.5). This mixture (0.05 ml) and 0.05 ml cell-free extract were incubated at 30 °C for 15 min, and the reaction was terminated by adding 2.0 ml of a ferric chloride/trichloroacetic acid reagent (Shapiro & Stadtman, 1970). The mixture was centrifuged at 3000 g for 20 min before the ΔAA<sub>540</sub> of the supernatant was measured against a reagent blank. Enzyme activity was expressed as ΔAA<sub>540</sub> min<sup>-1</sup> (10<sup>10</sup> cells)<sup>-1</sup>.
Analysis of total cell magnesium. Magnesium was analysed using flameless (electrothermal) atomic absorption spectrophotometry as described by Walker & Duffus (1980).

RESULTS AND DISCUSSION

In cultures of *S. pombe* and *K. fragilis* synchronized by induction using 2'-deoxyadenosine, marked fluctuations of glutamine synthetase activity and magnesium content occurred (Fig. 1). Similar results (not shown) were observed in synchronous cultures of both yeasts prepared by a magnesium-exhaustion method. These changes did not occur in an asynchronous control, and the exact pattern obtained depended on both the yeast and the method of synchronization. Although there may be a tendency for the enzyme activity to be high at the start of and during division, and low when the cell numbers are not increasing, this is not invariably so, and a much more convincing correlation exists between enzyme activity and cell magnesium content. These two values rise and fall together.

Sims et al. (1974b) have shown that glutamine synthetase activity in *Candida utilis* is largely controlled by variations in its rate of degradation. They suggested that Mg^{2+} could be an important modulator of this enzyme as it stabilizes the quaternary structure, protecting it against dissociation and proteolytic attack. Hachimori et al. (1974) have made similar observations with the enzyme from *Saccharomyces cerevisiae*. The results reported here would readily be explicable if glutamine synthetases from *S. pombe* and *K. fragilis* are also sensitive to changes in the concentration of magnesium.

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REFERENCES


