Effect of Ammonia on Glycogen Metabolism during Sporulation of *Saccharomyces cerevisiae*

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INTRODUCTION

Ammonia and glutamine inhibit sporogenesis of *Saccharomyces cerevisiae* (Durieu-Trautmann & Delavier-Klutcho, 1977; Miller, 1963; Piñon, 1977). These inhibitors have at least two sites of action, one related to premeiotic DNA synthesis, and another occurring later but not related to DNA synthesis (Durieu-Trautmann & Delavier-Klutcho, 1977; Piñon, 1977). Ammonia and glutamine also act on non-specific, though necessary, events in sporogenesis such as protein and RNA synthesis and breakdown (Durieu-Trautmann & Delavier-Klutcho, 1977).

Here we report the effect of ammonia on the synthesis and on the breakdown of glycogen, which are respectively non-specific and specific to sporogenesis.

METHODS

Yeast strains. Two diploid strains of *Saccharomyces cerevisiae* isogenic except for the mating type, 532 a/α and 533 α/α , were used (Pellucuer, 1973).

Growth and sporulation. Cells were grown in an acetate presporulation medium (Roth & Halvorson, 1969) containing (g l⁻¹ in 0.05 M-potassium phthalate pH 5.7): yeast nitrogen base, 6.5; ammonium sulphate, 5; potassium acetate, 10; and yeast extract, 1. After six generations, cells in exponential phase were transferred to a sporulation medium containing acetate (0.3 %, w/v) and raffinose (0.02 %, w/v) (Simchen *et al.*, 1972). In these conditions the first asci appeared at around 14 h and reached a level of 60 % at around 36 h. Where indicated, 5 mM-ammonia was added; it was removed by washing and placing the cells in fresh sporulation medium.

Determination of glycogen. Glycogen was extracted from samples containing 7×10^7 to 10×10^7 cells as described by Hopper *et al.* (1974); it was degraded to glucose by incubation with 1 mg amyloglucosidase (isolated from *Aspergillus niger*; 70 U mg⁻¹; Sigma) at 35 °C for 2 h. Glucose concentrations were determined using a Worthington glucostat kit (Boehringer).

RESULTS

In a/α sporulating cells (Fig. 1*b*), the initial level of glycogen was 0.05 mg glucose per 10^8 cells. The glycogen concentration increased during sporogenesis, reaching a maximum at 10 to 11 h, and then decreased; at 24 h the concentration was 59 % of the maximum. In α/α non-sporulating cells (Fig. 1*a*) the accumulation of glycogen was similar to that in a/α sporulating cells for the first 11 h, but no subsequent degradation was observed. These results agree with those of Kane & Roth (1974) and of Hopper *et al.* (1974), who reported that glycogen synthesis occurred in both sporulating and non-sporulating cells, but only in sporulating cells was it followed by glycogen breakdown.

When ammonia was added at 0 h to the sporulation medium, glycogen synthesis started in both strains, but it stopped suddenly at about 4 h; the glycogen concentration remained

Fig. 1. Effect of ammonia on the glycogen metabolism of non-sporulating a/a (a) and sporulating a/a (b) yeast cells placed in sporulation medium: control ($\circ$); ammonia added at 0 h ($\bullet$); ammonia added at 0 h and removed at 4 h (∇), at 7 h ($\square$) and at 11 h ($\triangle$); ammonia added at 11 h ($\blacktriangle$).

constant for the next 7 h and then gradually decreased. When ammonia was added at 0 h and withdrawn at 4 h, the changes in glycogen concentration were similar to those found in the untreated cells. When ammonia was withdrawn at 7 and 11 h, i.e. before the time of commitment of the cells to sporulation (Ganesan & Holter, 1958; Rothman & Cabib, 1969) which in our conditions was at 12 h (unpublished results), glycogen synthesis recommenced but the maximum levels were attained, respectively, 4 to 5 h or 8 h later than in untreated control cells. Ammonia still inhibited sporulation when it was added at 11 h; it also induced degradation of accumulated glycogen in a/a cells and accelerated this degradation in a/a cells.

DISCUSSION

In both a/a and a/a cells incubated in the presence of ammonia, glycogen synthesis was arrested at around 4 h, together with RNA and protein synthesis (Durieu-Trautmann & Delavier-Klutchko, 1977). The cessation of the glycogen synthesis caused by ammonia has already been described by Rothman & Cabib (1969), but in their experimental conditions, using yeast resting cells, the stoppage was sudden whereas it was delayed for 4 h in ours. These authors supposed the stoppage to be due to the arrest of synthesis. However, we observed that ammonia, added to the medium when the cell glycogen was at its highest, induced the degradation of glycogen in a/a cells and increased the degradation in a/a cells. Thus, ammonia acts on glycogen metabolism in the course of sporulation or of starvation but its effect depends on the time at which it is added.

It is noteworthy that ammonia has no effect on RNA, proteins (Durieu-Trautmann & Delavier-Klutchko, 1977) and glycogen synthesis occurring before 4 h, or on the initiation

of premeiotic DNA synthesis (Durieu-Trautmann & Delavier-Klutchko, 1977; Piñon, 1977).

We have shown elsewhere (Durieu-Trautmann & Delavier-Klutchko, 1977) that the inhibitory effects of ammonia on RNA and proteins synthesized after 4 h were reversible when the inhibitor was removed before the sporulating cells were committed; sporulation then recommenced at around 4 h and not at 0 h. Glycogen synthesis also restarted when ammonia was removed at 7 h, and at 11 h, but the maximum level was reached, respectively, 4 to 5 h and 7 to 8 h later and not after 7 h and 11 h as would be expected. Thus, in our conditions and with our strain, ammonia has no effect on the initiation of sporulation.

The inhibitory effect of ammonia on synthesis of macromolecules (RNA, proteins, glycogen) is not specific to sporulating cells. Sporulating cells, before commitment, and non-sporulating cells behave in the same way except for DNA synthesis and glycogen breakdown. Even the structural carbohydrates (mannan) necessary for spore wall construction are synthesized in the non-sporulating strain (Rothman & Cabib, 1969).

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