EFFECT OF AMMONIA AND GLUTAMINE ON MACROMOLECULE SYNTHESIS AND BREAKDOWN DURING SPORULATION OF SACCHAROMYCES CEREVISIAE

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SUMMARY. The effect of two known inhibitors of sporulation in yeast, ammonia and glutamine, on certain biochemical events during sporogenesis have been studied using sporulating a/α and non sporulating α/α cells. Both strains gave similar results on the increase in dry cell weight, protein and RNA breakdown and the suppression of the intensive RNA and protein syntheses occurring after 4 hours. The inhibitory effect of ammonia and glutamine on RNA and protein syntheses is reversible under the same conditions which do so for sporulation.

It is well known that sporogenesis is inhibited by ammonia (1) and glutamine (2 - 3). Some biochemical events such as premeiotic DNA synthesis (4) and glycogen breakdown (5 - 6) are specific for sporulation ; some, such as RNA and protein breakdown (5), are partially specific because they differ only quantitatively in sporulating and non sporulating cells ; while others, such as RNA, protein and glycogen synthesis, though necessary, are not specific since they have been observed to the same extent in both sporulating and nonsporulating cells (5 - 6). In the present study, we describe the effects of ammonia and glutamine on some of the above biochemical events in sporulating and nonsporulating strains of Saccharomyces cerevisiae.

METHODS

Two diploid strains, isogenic except for the mating type, 532 a/α and 533 α/α were used (7). Cultures were grown in a presporulation medium containing yeast nitrogen base, 6.5 g, ammonium sulfate, 5 g, and potassium acetate, 10 g, per liter of 0.5 mM potassium phtalate pH 5.7. After 6 generations, cells in exponential phase were transferred to a sporulation medium of potassium acetate and raffinose (8) ; under these conditions, the first asci appeared at about T_{16} and the final yield attained approximately 60% (3)(Fig. 2). When required 5mM ammonia or 10 mM glutamine were added. They were removed by washing and placing cells in fresh sporulation medium.

DNA was extracted according to the procedure of Roth and Silva-Lopez reported by Haber and Halvorson (9) and essayed colorimetrically using Burton modification of the diphenylamine reaction (10).

RNA and protein synthesis were measured by pulse labelling with [^{3}H] adenine, 2 µc/ml, and [^{14}C] leucine, 0.2 µc/ml, as described by Hopper and al. (5).

To determine RNA and protein breakdown, cells were grown with [^{3}H] adenine or [^{14}C] leucine and transferred to unlabelled sporulation medium.

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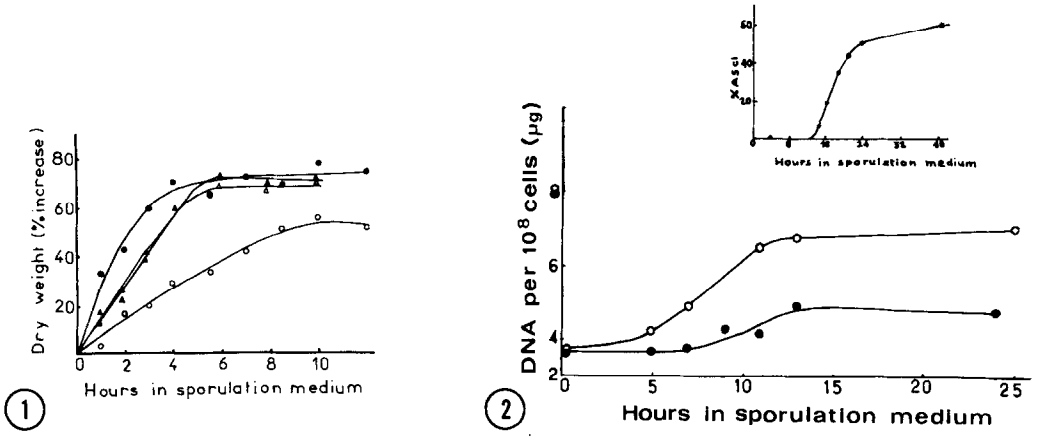


Fig. 1. Increase of dry cell weight
At T_0, weight of 10^8 a/∝ cells (o—o) was 2.1 mg, of 10^8 ∝/∝ cells (Δ—Δ) was 2.3 mg. Without inhibitor (o—o, Δ—Δ) and with inhibitor (●—●, ▲—▲).

Fig. 2. Effect of inhibitor on the kinetics of DNA synthesis in a/ cells during sporulation.
Without inhibitor (o—o), with inhibitor (●—●).

RESULTS

As identical results were obtained with ammonia and glutamine, only one curve will be presented for both inhibitors in each figure.

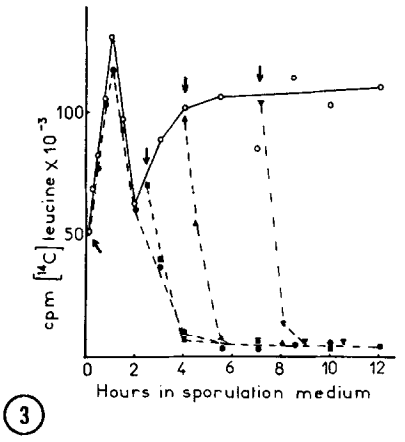
Cell number did not change appreciably during the course of experiments in all cases (a/∝ and ∝/∝ cells, with and without inhibitors).

Dry weight. In the absence of inhibitor, the dry weight of sporulating cells increased by 50% over the initial value (Fig.1). When sporulation medium contained ammonia or glutamine, the rise of dry cell weight was higher (70%). In cells unable to sporulate (∝/∝) the increase of dry cell weight was the same in the absence and in the presence of inhibitors (70%) and was similar to that observed with a/∝ in the presence of the inhibitor.

DNA synthesis. The effect of ammonia of glutamine on premeiotic DNA synthesis will be reported in detail elsewhere (3) and is indicated in Fig. 2 for the sake of comparison. In the presence of inhibitor, both the rate of DNA synthesis as well as the quantity were reduced.

Protein synthesis. In the absence of inhibitor a clear peak of protein synthesis was observed around T_1*, followed by a rise at T_4 which

*T_1, T_2, T_3.... : 1,2,3.... hours after transfer of the cells to sporulation medium



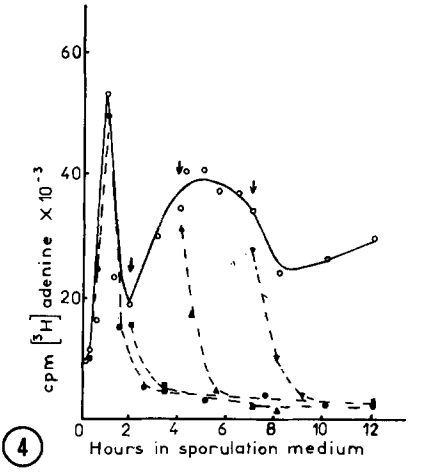


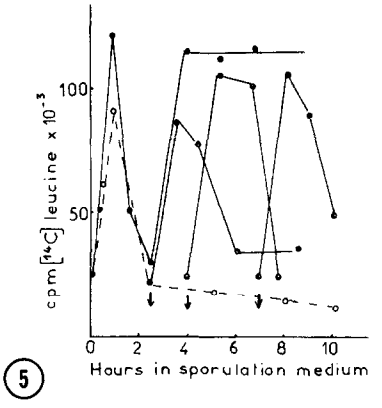
Fig. 3. Protein synthesis in a/α or α/α cells.
Without inhibitor (solid lines) and with inhibitor (broken lines).
Inhibitor was added at the time shown by the arrows. 4×10^6 cells were used for counting.

Fig. 4. RNA synthesis in a/α or α/α cells.
Same legend as in Fig. 3.

was maintained until at least T_{12}. Addition of ammonia or glutamine to the sporulation medium at T_0 or $T_{2.30}$ prevented the second increase of [^{14}C] leucine incorporation into protein. Addition of the inhibitors at T_4 or T_7 stopped protein synthesis and the rate of [^{14}C] leucine incorporation declined rapidly. Similar results were obtained with both strains a/α and α/α (Fig. 3).

RNA synthesis. In both strains a/α and α/α, RNA synthesis varied throughout incubation in sporulation medium (Fig. 4) and showed two main periods of incorporation of ^{3}H adenine : a sharp peak between $T_{0.5}$ and T_1, and a second broad one after T_4. When ammonia of glutamine was added at T_0 or $T_{2.5}$ the second period of RNA synthesis was prevented ; when added at T_4 or T_7 RNA synthesis was immediately depressed.

Reversibility of protein and RNA synthesis. Inhibitor was added at T_0 and removed at $T_{2.50}$, and T_7 respectively. Results illustrated in Fig. 5 and 6, show an increase of [^{14}C] leucine incorporation, or [^{3}H] adenine incorporation into protein or RNA fractions after the removal of inhibitor, whatever the time of this removal. This increase was followed by a decline approximately three hours later.



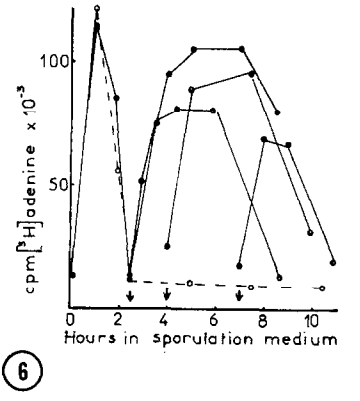


Fig. 5. Effect of inhibitor removal on protein synthesis in a/α cells or α/α cells.
Control cultures without inhibitor (●—●) and with inhibitor (○—○).
Inhibitor was removed at the times indicated by arrows.
4 x 106 cells were used for counting.

Fig. 6. Effect of inhibitor removal on RNA synthesis in a/α cells or α/α cells.
Same legend as in Fig. 5.

Protein and RNA breakdown. As described by Hopper and al. (5), protein and RNA breakdown occurred more extensively in a/α than in α/α cells. In cells submitted to the inhibitors, breakdown increased approximately three-fold in all cases (data not shown).

DISCUSSION

The aim of this work was to determine the effect of ammonia and glutamine, which inhibit sporogenesis, on some of the main events involved in sporulation.

We have shown previously (3) that ammonia and glutamine have at least two sites of action, one functioning early around T_4, probably related to DNA synthesis, and a later one between T_4 and T_{12} not related to it. The inhibitors however do not affect the initiation of premeiotic DNA synthesis which takes place before T_4. In the present work, we have observed that neither inhibitor acts on the protein and RNA syntheses which occur before T_4 ; but both prevent the syntheses occurring after T_4. Thus, it seems that, in our conditions and with our strains, inhibitors have no effect on the initiation of sporulation. Moreover when inhibitors added at T_0 are removed later than

T_4, sporogenesis recommences at about T_4 (3) and parallely RNA and protein syntheses, which were blocked, increase again.

Protein and RNA syntheses occurring after T_4 and sensitive to the action of inhibitors are necessary events ; but are probably not endowed with a certain specificity since similar results are obtained with nonsporulating α / α cells.

Protein and RNA breakdown are higher in cells submitted to the inhibitors than in untreated cells. The intensive release of $[^{14}C]$ leucine and $[^{3}H]$ adenine into the medium may be due to the non utilisation of these precursors and may also explain the brief synthesis of RNA and protein which follows the removal of inhibitors.

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