Cell Death Produced by Antibiotic Cerulenin in Biotin-requiring Yeast Saccharomyces cerevisiae

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In the biotin-requiring yeast Saccharomyces cerevisiae, death due to unbalanced growth occurs when cells are cultured in biotin-free medium containing aspartic acid (Asp medium) or its amide as nitrogen source, and in this case, dead cells have an undeveloped bud lacking a nucleus. Such death from unbalanced growth has similarly been observed in pantothenate- or inositol-starved yeast cells. Fatty acid mutants of Saccharomyces cerevisiae were found to die when deprived of required fatty acids. It has been suggested that death may result from defective membranes because biotin, pantothenate or inositol affect lipid synthesis. Recently, Mizunaga et al. reported in relation to cell death in biotin-requiring yeast S. cerevisiae that the primary cause of death in biotin deficiency was assumed to be insufficient synthesis of endogenous long-chain fatty acids in cells grown on Asp medium. However, it is still not directly evident whether the prime cause of cell death is the prevention of fatty acid-containing lipid synthesis under biotin deficiency.

In order to demonstrate the direct relationship between cell death and lipid synthesis in biotin-requiring yeast, we employed the antibiotic cerulenin. This antibiotic has been shown to specifically inhibit the biosynthesis of fatty acids and sterols in yeasts. The present communication describes the newly constructed conditions of adding cerulenin to produce a higher percentage of dead cells, and under these conditions, the effect of cerulenin on incorporation of acetate into cell lipids.

Baker's yeast, Saccharomyces cerevisiae strain BA-1 used here was cultured in synthetic medium, as previously described. In order to produce a higher percentage of dead cells than in Asp medium without biotin, S. cerevisiae grown on biotin-deficient medium containing ammonium sulfate (Am medium) for 32 hr was harvested, washed once with sterile deionized water, and then inoculated into biotin-sufficient medium containing aspartic acid (Asp medium) and various amounts of cerulenin. The Asp medium used here contained 0.002 μg/ml biotin. In this medium without cerulenin, biotin-deficient cells fully recovered to normal growth. For the labelling experiment, cells grown on Am medium, as described above, were collected, washed, resuspended in Asp medium containing various amounts of cerulenin plus biotin, and incubated for 2.5 hr. Two and half hours later, 0.2 μCi of [U-14C]sodium acetate per ml was added to the above medium, and cells were further incubated for 1 hr. At the time indicated, duplicate 1.0-ml samples were removed from the culture, added to 5.0 ml of ice-cold 5% trichloroacetic acid, and immediately centrifuged. The washed cells were resuspended in 30 ml of chloroform-methanol (2:1, v/v) and mechanically disrupted with glass beads in a Braun homogenizer for 4 min. Lipids were extracted from the disrupted cells by the method of Letters.

As shown in Fig. 1, growth was almost inhibited by the addition of 1.0 μg or more cerulenin per ml, inhibited partially by 0.4 μg/ml, and inhibited slightly by 0.2 μg/ml (curve A). On the other hand, rapid cell death occurred in 0.2 μg/ml of cerulenin, and the maximum rate of cell death was achieved when 0.4 μg/ml cerulenin was present in growth medium (curve B). At this concentration, there also remained a constant higher percentage of dead cells compared with the case in Asp medium without biotin. This death, however, was partially prevented by increasing...
FIG. 2. Time Course of the Effect of Cerulenin on Cell Growth (A) and Death (B).

Deficient cells of S. cerevisiae were inoculated into Asp medium containing no cerulenin (○); no cerulenin and 0.002 µg of biotin per ml (•); and 0.4 µg of cerulenin and 0.002 µg of biotin per ml (△). After 8 hr, portions were removed, and cell growth and percentage of dead cells were measured.

the amount of cerulenin, suggesting that the partial prevention of cell death was due primarily to growth inhibition in higher concentrations of cerulenin. Figures 2A and B show the time course of the effects of 0.4 µg cerulenin per ml on cell growth and death. When cells were grown in Asp medium containing cerulenin (0.4 µg/ml) plus biotin, cell growth became repressed to the growth level of control cells in Asp medium lacking biotin (Fig. 2A). In medium supplemented with cerulenin, cells also began to die after 3-hr incubation. Cell death in cerulenin-supplemented medium became reached to the level of death in biotinless medium during the subsequent 5 hr period (Fig. 2B). Cell death in the presence of cerulenin resulted in the same rate as in biotinless culture over a period of 8 hr. In addition, most cells had a bud as they died, and light microscopy revealed to shrink to about one-half their size as control cells (data not shown). These findings imply that addition of cerulenin to biotin-sufficient Asp medium also resulted in a similar pattern of cell death produced by removal of exogenous biotin from Asp medium. Therefore, as mentioned above, such conditions provoking cell death are considered useful for examining the mechanism of death due to biotin deficiency.

In yeasts, both fatty acid synthesis and sterol synthesis were found inhibited by cerulenin. It was of interest to determine whether cell death was due to the specific inhibition of lipid synthesis by cerulenin. In

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FIG. 3. Effect of Cerulenin on Lipid Synthesis.

Cells precultured in Am medium were distributed into Asp medium containing various amounts of cerulenin in the presence of biotin. Two and half hours later, 0.2 µCi of [U-14C]sodium acetate was added to medium, and 1 hr later, samples were removed. Incorporation of radioactivity into cell lipids was measured. Asp medium containing biotin, lipid synthesis measured by incorporation of [14C]acetate into lipid fraction extracted from disrupted cells was inhibited by the addition of cerulenin. Lipid synthesis at a concentration of 0.2 µg/ml cerulenin was slightly inhibited and partially inhibited at 0.4 µg/ml. Also at a concentration of 0.4 µg/ml cerulenin, the incorporation of acetate into the cell lipid fraction was reduced to nearly 70% of control cells in Asp medium without cerulenin (Fig. 3). Higher concentrations of cerulenin caused greater inhibition of lipid synthesis, resulting in about 7% of the control level. The result obtained here is in agreement with Mizunaga et al. and shows that cell death results from partial inhibition of lipid synthesis caused by antibiotic cerulenin (i.e., an insufficient synthesis of fatty acid-containing lipids), implying a direct correlation between cell death and lipid synthesis. Furthermore, this is the first observation on the effect of cerulenin on death of yeast cells budding in the presence of biotin.

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