Combination of 2-Deoxyglucose and Snail-gut Enzyme Treatments for Preparing Sphaeroplasts of Schizosaccharomyces pombe

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Havelkova (1966), Rost (1969) and Shahin (1971) isolated sphaeroplasts from Schizosaccharomyces pombe by using snail-gut enzymes. However, yields were usually low, and certain strains were not sensitive to snail-gut enzymes especially when grown in glucose-containing medium. Sphaeroplasts of Schiz. pombe can be obtained by using 2-deoxyglucose (2-DG) (Megnet, 1965; Johnson & Rupert, 1967; Johnson, 1968; Johnson, 1969). Recently Birnboim (1971) described a new method for preparing sphaeroplasts of Schiz. pombe 972 h-1 at 0 °C by snail-gut enzymes after prior adaptation to 70 µg 2-DG/ml by subculturing the cells daily into medium containing increasing concentrations of 2-DG. Using this method, 10^7 organisms/ml can be treated by 1 % snail-gut enzyme.

In this paper, we report that in Schizosaccharomyces pombe, sphaeroplasts obtained by 2-DG lose their respiratory capacity. It appears that long treatments with 2-DG increase the risks of possible metabolic perturbations as well as selection of 2-DG-resistant clones. However, short pre-incubation of growing organisms with 2-DG has no influence on respiration, and greatly facilitates subsequent hydrolysis of walls of four different strains of Schiz. pombe by snail-gut enzymes. Under these conditions 10^8 organisms/ml can be efficiently treated with 1 % snail-gut enzyme.

METHODS

The YPDG medium contains: 3·6 % (w/v) glycerol, 0·1 % (w/v) glucose, 2 % (w/v) yeast extract, 2 % (w/v) bactopeptone. The GLU medium contains: 5·8 % (w/v) glucose, 2 % (w/v) yeast extract, 2 % (w/v) bactopeptone. All media were brought to pH 4·5 with HCl. To prepare sphaeroplasts of Schizosaccharomyces pombe 972 h^-1, 100 µg 2-DG/ml (in YPDG medium) or 250 µg 2-DG/ml (in GLU medium) were included in the medium of cultures at the end of exponential phase of growth. Organisms were harvested 20 min later in GLU medium and 30 min later in YPDG medium, before sphaeroplasts were visible. Organisms, washed twice with cold sterile water, were incubated at room temperature with continuous shaking for 30 min in 0·1 M-tris-HCl (pH 8·0), 0·5 M-sorbitol and 0·3 M-β-mercaptoethanol. After centrifugation at 2500g for 10 min, they were diluted to 10^9 organisms/ml in 1 M-sorbitol and incubated at 30 °C with continuous shaking, in the presence of 1 % gluculase (Endo Laboratories, Garden City, New York, U.S.A.) or 1 % helicase (Industrie Biologique Française, Villeneuve-la-Garonne, France). The rate of the enzyme action was measured by lysis in samples diluted with water. Organisms were harvested and washed twice in 1 M-sorbitol, when 75 % of them were transformed in sphaeroplasts.
Short communication

RESULTS AND DISCUSSION

Preparation of sphaeroplasts by 2-DG. When added at the beginning of the culture, 2-DG inhibited growth of Schizosaccharomyces pombe 972 h⁻¹. In 24 h, 1 μg of 2-DG/ml inhibited growth by 50% in glycerol-containing medium. The same degree of inhibition required 100 μg/ml in glucose-containing medium. Addition of 2-DG to media at the end of the exponential phase of growth allowed rapid inhibition of growth, followed by sphaeroplast formation both in glucose- and glycerol-containing media. In glycerol-containing medium addition of 0.15% glucose just before introduction of 2-DG greatly speeded the sphaeroplast formation. Two hours after addition of 100 μg 2-DG/ml plus 0.15% glucose to Schiz. pombe 972 h⁻¹ growing in glycerol-containing medium, 95% of the organisms were converted to sphaeroplast but the respiration rate dropped from 200 to 40 μl O₂ h⁻¹ (10⁶ cells)⁻¹. Similar decreases in respiratory rate were observed under every condition producing sphaeroplasts by the sole use of 2-DG.

Combined actions of 2-DG and snail-gut enzyme

Short pretreatment with 2-DG largely amplified subsequent attack by snail-gut enzyme on organisms grown in the presence of glucose or glycerol (Fig. 1). However specific conditions of the 2-DG pretreatment and snail-gut enzyme treatment had to be carefully controlled. The 2-DG pretreatment had to make the walls fragile, without leading to lysis and causing inactivation of respiration. Therefore, it has to be adapted for each strain and each culture condition. For the wild-type strain, pretreatments for 20 min with 100 μg 2-DG/ml (YPDG medium) or 30 min with 250 μg 2-DG/ml (GLU medium) were sufficient. Subsequent
treatment with β-mercaptoethanol was always beneficial for sphaeroplast formation. Glusulase was more efficient than helicase. The optimal density during enzyme treatment was about 10⁹ organisms/ml. Above this density the efficiency of the enzyme decreased, and the use of higher enzyme concentrations did not compensate. At enzyme concentrations higher than 1% sphaeroplasts tended to burst prematurely during enzyme treatment, despite the presence of 1 M sorbitol. In order to avoid premature bursting, the enzyme treatment had to be stopped when sphaeroplast counts reached about 75% of the total. Under these conditions, sphaeroplasts obtained by the combined actions of 2-DG, β-mercaptoethanol and glusulase exhibited the same respiratory rate as intact organisms.

**Induction of sphaeroplasts from mutants**

The method described above was tested on four mutants modified in their respiratory properties: m126, m53 (Goffeau, Colson, Landry & Foury, 1972), c085 and c086 (Foury & Goffeau, 1972). 2-DG induced wall lysis of all mutants except c086. However, this last strain was very sensitive to glusulase and 2-DG pretreatment was not needed. For the other strains, 2-DG pretreatment markedly facilitated the action of glusulase. After 2-DG pre-treatment, the percentage of sphaeroplasts formed by 1 h treatment with 1% glusulase, increased from 5 to 30% for m126, from 5 to 36% for m53 and from 25 to 85% for c085. Specific conditions of the 2-DG pretreatment had to be adapted to each mutant; 100 µg 2-DG/ml for 30 min was effective for glucose-grown m126, whereas 500 µg 2-DG/ml for 60 min was required for glucose-grown m53 and c085. Short 2-DG pretreatment also facilitated sphaeroplast formation in glycerol grown-cells.

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**REFERENCES**


