Induction of Respiratory-Deficient Nonchromosomal "Petites" of Saccharomyces cerevisiae by Sodium Dodecyl Sulfate

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Received for publication 3 April 1973

Nonchromosomal "petites" can be produced in Saccharomyces cerevisiae by treatment with sodium dodecyl sulfate, an anionic surface active agent.

A number of chemical agents have been found to induce the respiration-deficient mutation in yeast (6, 14, 16). Two types of mutants can be considered in two genetic classes: (i) the chromosomal mutants ("segregational mutants") and (ii) the cytoplasmic or ρ- mutants (vegetative "petites"). The ρ- are (i) completely deficient in cytochromes a and aₕ; (ii) either completely or almost completely deficient in cytochromes b and c; and (iii) have normal or slightly higher amounts of cytochrome c. In addition, the mitochondria are generally non-functional and display structural abnormalities (18). In the present work, sodium dodecyl sulfate (SDS) was shown as an inducer of respiratory deficiency of yeast mitochondria by a nonchromosomal mode of inheritance.

For these experiments, SDS (Sigma Chemical Co.) was analyzed by emission spectroscopy (Perkin-Elmer, model 303). In addition to sodium, which was the major metallic component, calcium (0.7 ppm), magnesium (0.1 ppm), and copper (less than 0.01 ppm) were present as impurities.

The respiring yeast Saccharomyces cerevisiae (strain 1203, W. Sloof, Centralbureau Voor Shimmelcultures, Delft, Netherlands) was inoculated in the yeast-peptone-dextrose medium containing dehydrated yeast extract (9.6%) (Difco), peptone (0.8%) (Difco), dextrose (2.0%), and SDS at various concentrations. The subsequent inocula were incubated aerobically at 30 C. Viable counts were carried out at intervals of 24 h by using the triphenyltetrazolium chloride overlaying technique of Ogur, John, and Nagai (8). The appearance of the mutants occurs in the SDS concentration range of 7.0 × 10⁻³ to 3.5 × 10⁻¹ M, which is in between the concentration possessing total lethal effect (8.0 × 10⁻² M) and the one showing low efficiency (2.0 × 10⁻¹ M), on a population of about 10⁴ cells/ml (Table 1).

Unlike acriflavine and ethidium bromide, SDS shows an immediate and high lytic effect on yeast cells (Fig. 1). However, SDS does not induce a "petite" mutation so efficiently as displayed by these compounds. A few hours after the addition of SDS, the number of "petite" cells (initial counting: 1.8 × 10⁴ cells/ml) began to raise slowly, becoming exponential after 24 h of incubation and reaching 1.5 × 10⁸ cells/ml, after 48 h of growth.

In a yeast-peptone-glucose medium (glycerol 3% instead of dextrose), the production of "petite" mutants began immediately after the addition of SDS and continued until the twentieth hour of culture (Fig. 2). Glycerol medium specifically selects against the growth of "petite" cells, so that could only have increased their number by induction during the growth of respiratory competent cells (5).

Mutants obtained by the action of SDS do not oxidize nonfermentable substrates (acetate, lactate, and glycerol) and do not show any respiratory activity as was determined by an oxygen electrode, by using ethanol as substrate. A lack of endogenous respiration was also observed.

A difference spectrum carried out between a suspension of "petite" cells chemically reduced with sodium dithionite and a suspension of mutant cells saturated with atmospheric oxygen strongly indicated the absence of cytochromes a and aₕ and probably b and c₁.

Crosses of 10 haploid strains of SDS "petite" mutants obtained from the strain D 585-11C (α lys), with chromosomal "petite" mutants of an opposite mating type D 602-1A (α pet, ρ⁺ lys, his₃ trp₃) in each case yielded hybrid cells which could respire and grow on glycerol medium.
TABLE 1. Occurrence of “petite” cells of S. cerevisiae by treatment with various concentrations of SDS

<table>
<thead>
<tr>
<th>SDS concn (M)</th>
<th>Viable* count (cells/ml)</th>
<th>Proportion of “petite” cells to normal colonies</th>
<th>Frequency “petite” colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 x 10^-3</td>
<td>0</td>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>9.0 x 10^-3</td>
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<td>7.0 x 10^-3</td>
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<td>4:0</td>
<td>100.00</td>
</tr>
<tr>
<td>6.0 x 10^-3</td>
<td>1.5 x 10^2</td>
<td>15:0</td>
<td>100.00</td>
</tr>
<tr>
<td>5.5 x 10^-3</td>
<td>5.5 x 10^2</td>
<td>54:1</td>
<td>98.20</td>
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<td>97.50</td>
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<td>225:204</td>
<td>52.40</td>
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<td>248:338</td>
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<td>132:859</td>
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<td>2.4 x 10^6</td>
<td>154:2452</td>
<td>5.90</td>
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<td>6.7 x 10^8</td>
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<td>0.88</td>
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<tr>
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<td>4.0 x 10^9</td>
<td>3:403</td>
<td>0.74</td>
</tr>
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<td>2:570</td>
<td>0.35</td>
</tr>
<tr>
<td>0*</td>
<td>3.0 x 10^11</td>
<td>1:800</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Viable counts were determined by triphenyltetrazolium chloride overlaying technique (9). The size of the initial inoculum in the medium was 2.0 x 10^4 cells/ml. The time of incubation was 24 h. It can be seen that a slightly inhibiting effect appears already at a low level of SDS concentration. However, since the spontaneous frequency of mutation with low concentrations of SDS (9.0 x 10^-4 to 2.0 x 10^-8 M) is less than 1%, it is possible to consider an inducing effect only when it shows values above 6%.

Average value of six experiments.

This and the high frequency of “petite” induction, as described before, indicate that SDS-induced mutants were of the ρ^- type.

Acriflavine, ethidium bromide, and SDS are known to have the effect of eliminating F or R factors, as well as penicillinase plasmids, in bacteria (1-4, 15, 17, 20). Also, they give rise to the production of “petite” mutants in yeast. The similarity of action displayed by these compounds on the extrachromosomal elements in bacteria and on the genetic components of mitochondria, the finding that mitochondrial deoxyribonucleic acid (DNA) has a bacterial-like organization (19), and the similarity between the structure and the replication of mitochondrial DNA and the bacterial DNA (7, 9, 12) could probably correlate the genetic determinant in yeast with bacterial plasmids, as well as with the evolutionary origin of mitochondria (10, 11).

Several other denaturant agents such as sodium lauryl sarcosinate, sodium palmitoyl sarcosinate, and guanidine hydrochloride also act as inducers of “petite” mutants from yeasts (unpublished data).

FIG. 1. Lytic effect of SDS on Saccharomyces cerevisiae cells and isolation of “petite” mutants. Sample of a SDS solution to make up a final concentration of 6.9 x 10^-4 M was transferred to a yeast peptone dextrose liquid medium containing 3.4 x 10^8 cells/ml. Immediately after the addition of SDS to the cell suspension, a sample of the whole system was taken for viable counting and determination of “petite” mutants at the zero time. Immediately after this, the system was incubated at 30 C. Samples were taken during 48 h, diluted, and plated in yeast peptone dextrose liquid medium, incubated for 48 h at 30 C and “petite” colonies were shown through the triphenyltetrazolium chloride test (curve b). After some minutes of SDS addition, a sudden decrease of normal cell viability was observed (curve a). On the other hand, after the first few hours of SDS addition, the number of “petite” cells began to increase slowly from 1.8 x 10^11 cells/ml, reaching 3.5 x 10^15 in 12 h. After 24 h of incubation, the production of “petite” cells became exponential, reaching 1.5 x 10^18 cells/ml after 48 h of incubation.

It can be supposed that SDS prevents the synthesis of one or two critical proteins important to the integrity of certain structures, which then leads to a variety of secondary defects. Alternatively, the SDS treatment could lead to the physical elimination of some cytoplasmic DNA maybe due to some membrane alteration meaningful for certain activities of the mitochondria.
This investigation was carried out with a grant in aid from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Projeto FUNTEC no. 51, Banco Nacional do Desenvolvimento Econômico.

LITERATURE CITED


