Evidence for a Surface Protein Layer on the Saccharomyces cerevisiae Ascospore

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Electrophoretic measurements on Saccharomyces cerevisiae ascospores indicated the presence of a surface protein layer which can be removed by papain, chymotrypsin or 8 M urea.

Little is known about the composition of the outer layers of the yeast ascospore. Because of the hydrophobic nature of yeast ascospores and their affinity for Sudan Black, several workers (3, 9, 13) suggested that the outermost layer is composed of lipid. This conclusion is not consistent with the presence, on the outside of all yeast ascospores so far examined (5, 8), of an electron-dense layer or with the marked ultraviolet-absorbing properties of this layer (12). This report deals with the electrophoretic properties of ascospores from Saccharomyces cerevisiae before and after treatment with various reagents. The data suggest that the yeast ascospore is covered with a layer of protein which overlays a thick spore wall probably composed of polysaccharide.

The strain of S. cerevisiae (DCL 740) was grown in the presporulation (nutrient broth plus 5% glucose and 1% yeast extract) and KCl (1.0%)-sodium acetate (0.5%) sporulation media recommended by Fowell (5). Approximately 60 to 65% of the cells sporulated after 5 days of incubation at 25°C in the sporulation medium. Ascii and vegetative cells were harvested by centrifugation at 0°C and washed twice with water. A suspension of cells and asci (80 mg, dry weight, per ml of 50 mM sodium acetate buffer; pH 5.5) was supplemented with one-third volume diluted snail juice (7) and incubated at 30°C for 24 hr. The cells and asci were harvested, and the ascospores were released from asci by subjecting a cold-water suspension to sonic treatment for 3 min with an ultrasonic disintegrator (Measuring & Scientific Equipment, Ltd.) at 20 kc per sec. Release of ascospores from asci was monitored by microscopic examination.

Figure 1 shows the pH-mobility curve for untreated ascospores. The shape of the curve is indicative of an amino-carboxyl surface, probably of protein (14). Further evidence against the presence of a lipid surface came from the finding that the mobility of spores was not affected by incorporating sodium dodecyl sulfate (0.1 to 0.001 mM) in 0.01 M phosphate buffer (pH 7.0) (4). Digestion of spores with trypsin did not alter the shape of the pH-mobility curve, although it increased the mobility values at high and low pH values. Digestion with pepsin or chymotrypsin changed the electrophoretic mobility pattern to one characteristic of a negatively charged surface (Fig. 2). A similar effect was produced after treatment of isolated spores with 8 M urea (Fig. 2). Electron micrographs of thin sections through ascospores showed that 8 M urea completely removed the electron-dense layer surrounding the spores. Ascospores which had been treated with papain or chymotrypsin retained small amounts of electron-dense material on the surface. These data suggest that the outside of the ascospores from S. cerevisiae is coated with protein, a conclusion which is in agreement with the electron-dense (5.8) and ultraviolet-absorbing (12) properties of this layer. However, the data do not preclude the possibility that the outer layer is composed of a lipoprotein, the lipid moiety of which lies below the surface of the spore. The hydrophobic character of the yeast ascospore suggests that the surface protein may resemble the structural protein found in mitochondrial membranes (1). Marquardt (10) reported that the outside layer of the yeast ascospore is synthesized by the ascal protoplasm rather than by the spore. The electrophoretic mobility of ascospores after digestion with pepsin or chymotrypsin, or treatment with 8 M urea, suggests that the underlying electron-transparent spore wall is probably composed of polysaccharide which is not covalently linked to the surface protein. Eddy and Rudin (2) showed that
residues in the cell-surface mannan (11). However, stationary-phase cells and asci of S. cerevisiae DCL 740 have no net charge which suggests that their surface layers differ in composition from the ascospore wall. The known variability of mannose:phosphate ratio in the mannans of different strains and species of yeast could well account for the lack of charge in DCL 740.

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**LITERATURE CITED**


