Stability of the Plasma Membrane in Saccharomyces rouxii and Its Relationship to Glucose Tolerance

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The stability of spheroplasts from the osmotrophic yeast Saccharomyces rouxii was studied in buffered solutions of mannitol and glucose. The plasma membranes from cells grown in high glucose concentrations were more stable to osmotic lysis than were membranes from cells grown in lower glucose concentrations. Mannitol was a better osmotic stabilizer than glucose, except when the cells were grown in a high glucose concentration. Spheroplasts from a glucose tolerant-deficient mutant were much less stable than the corresponding spheroplasts from the parent strain, especially when suspended in glucose solutions. These results suggest an involvement of the plasma membrane in the glucose-tolerant mechanism of S. rouxii.

Many foods are preserved by drying or by the presence of large amounts of solutes, employing the concept that removal or unavailability of water will make the food resistant to microbial spoilage. These are the types of food in which osmotrophic yeasts can be important spoilage agents. The osmotrophic yeast Saccharomyces rouxii is a unique microorganism since it is tolerant to high concentrations of both sugars and sodium chloride. It can also grow quite readily in dilute media (9, 16). A limited number of studies have been made of the mechanism of solute tolerance of this organism. Brown has proposed that the intracellular accumulation of D-arabitol is involved in sugar tolerance (3, 4) and that the accumulation of glycerol is involved in salt tolerance (8). It has become increasingly clear that different mechanisms are involved in sugar and salt tolerance (8, 16) and also that different mechanisms are involved for different sugars (15). We have recently reported that S. rouxii has an active pentose phosphate pathway, which was not observed in a glucose tolerant-deficient mutant (15).

The idea of the involvement of the plasma membrane in sugar tolerance has been disregarded by previous investigators due to the presence of a thick cell wall in yeasts (4). We have observed the inability of whole cells of a glucose tolerant-deficient mutant to withstand a relatively mild osmotic shock (15). Shrinkage of S. rouxii cells has been observed when they were suspended in hypertonic solutions of various solutes, but the shrinkage was less than that observed with a nonsmotrophic strain (6, 18). Plasmolysis was also very slight in S. rouxii when compared with other organisms, although it was not compared with a nonsmotrophic yeast (6, 7). These observations suggest that the ability of the membrane of S. rouxii cells to stretch and contract may be involved in its solute-tolerant properties.

The chemical composition of biological membranes influences their physical properties of expansion and contraction (17). Koh (13) found that the chemical composition of the cell envelope of S. rouxii was changed by growth in high concentrations of sucrose. In this study, we have presented evidence that the stability of S. rouxii plasma membranes is altered by the concentration of glucose in the growth medium.

S. rouxii NRRL Y-1600 was maintained and grown aerobically in a complex medium as previously described (15). A mutant, which was unable to grow in a 60% glucose (wt/vol) medium, was derived from strain Y-1600 after ethyl methane sulfonate mutagenesis and designated Glt4 (15). Concentrations of 1, 30, or 60% (wt/vol) glucose were used in the growth media.

Cells were grown to logarithmic phase, harvested, and washed as previously described (15). The cells were suspended to an approximate concentration of 20% (wt/vol) in a medium containing 10 mM dithiothreitol, 1.0 M mannitol, and 100 mM sodium phosphate buffer (pH 6.8) and preincubated for 30 min at 30°C (2, 5). The preparation used to digest the cell walls was a crude solution from the intestine of Helix pomatia (β-glucuronidase, type H1, Sigma Chemical Co.) which had been diluted 10-fold in the above medium. The preincubated cells were mixed with an equal volume of the diluted snail enzymes and incubated for 1.5 h at 30°C with occasional shaking. Spheroplast formation was
followed microscopically, and 95% of the cells were converted to spheroplasts after 1.5 h. The spheroplasts were harvested by centrifugation at 9,300 × g for 5 min and washed twice in phosphate buffer with 1.0 M mannitol. The mutant strain, Glt4, was stabilized with 1.2 to 1.5 M mannitol. The spheroplasts were resuspended in the stabilized buffer to 15% (wt/wt), based on the starting yeast concentration.

The method used for determining the relative stability of *S. rouxii* spheroplasts was essentially that which was originally described by Altemus and Rose (1) and subsequently used by Hossack and associates (10, 11). The starting spheroplast suspension contained approximately 25 mg (dry weight equivalence) per ml of buffered mannitol and had an initial absorbance of about 0.7 at 500 nm. Each tube was blanked against the corresponding concentration of mannitol or glucose to correct for the change in absorbance due to the change in concentration of the solution.

The stabilities of the plasma membranes of the parent strain suspended in solutions of mannitol are shown in Fig. 1a. As the concentration of glucose in the growth medium was increased, the stability of the plasma membranes of the cells to the lysis in mannitol was increased. The stabilities of the spheroplasts suspended in glucose are shown in Fig. 1b. The same trend was observed: the stability of the spheroplasts increased when the cells were grown in higher glucose concentrations. Whereas the stability of the membranes from cells grown in 60% glucose was similar in mannitol and glucose, it was obvious that the comparative stability of the membranes from cells grown in 1 and 30% glucose was much less when the cells were suspended in glucose. Apparently, polyalcohols, such as mannitol, help stabilize the plasma membranes of *S. rouxii* from osmotic lysis.

The stability of the membranes from the mutant strain was examined by plotting the optical density readings with the corresponding data from the parent strain. The stability in mannitol of the membranes from the mutant strain grown in 1% glucose is shown in Fig. 2a, and the stability when the cells were grown in 30% glucose is shown in Fig. 2b. In both cases, the membranes of the mutant strain were less stable than those of the parent strain. When the spheroplasts were suspended in glucose, the initial stability of the membranes from the mutant strain was much less than that of membranes from the parent strain and was also less than that observed in mannitol for 1%-grown cells (Fig. 2c). Even greater differences between the mutant and parent, through the whole range of glucose concentrations, were observed when membranes from cells grown in 30% glucose were measured (Fig. 2d). The membranes of the mutant strain were very sensitive to osmotic lysis in glucose solutions, and this sensitivity was most remarkable when the cells were grown in 30% glucose.

The data from these experiments indicate that *S. rouxii* has a mechanism for strengthening its membranes against osmotic lysis when grown in concentrated solutions of glucose; the mutant strain appears to have lost the ability to strengthen its membranes. Another important observation from this study was that the polyalcohol mannitol was a better membrane stabilizer than glucose. Mannitol has been recommended as being more efficient than glucose in stabilizing yeast spheroplasts (14); this may be due in part to the metabolic lysis of yeast spheroplasts.

**Fig. 1.** Stability of spheroplasts from cells of *S. rouxii* NRRL Y-1600 grown in different concentrations of glucose and suspended in buffered solutions of (a) mannitol or (b) glucose. Symbols: ○, cells grown in 1% glucose; ●, cells grown in 30% glucose; △, cells grown in 60% glucose.
caused by glucose (12).

We have previously shown that the production of another polyalcohol, δ-arabitol, in S. rouxii was related to the presence of an active pentose phosphate pathway (15). The production of δ-arabitol served as a fermentation product which allowed the regeneration of oxidized nicotinamide adenine dinucleotide phosphate as well as a solute compatible with enzyme function (4). This pathway would ensure a large supply of reduced nicotinamide adenine dinucleotide phosphate which could be used for lipid synthesis and modification of the plasma membrane (19). Modifications in the chemical composition of the plasma membrane may help account for the results reported in this study.

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**Fig. 2.** Comparison of the stability of spheroplasts from cells of S. rouxii NRRL Y-1600 (○) and its glucose tolerant-deficient mutant, strain Glt4 (●). Cells were suspended in buffered solutions of mannitol after growth in (a) 1% glucose or (b) 30% glucose, or cells were suspended in buffered solutions of glucose after growth in (c) 1% glucose or (d) 30% glucose.

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