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Stability of the Plasma Membrane in *Saccharomyces cerevisiae* Enriched with Phosphatidylcholine or Phosphatidylethanolamine

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Spheroplasts from *Saccharomyces cerevisiae* NCYC 366, enriched in phosphatidylethanolamine after growth in medium supplemented with 1 mM ethanolamine, were more resistant to osmotic lysis than were spheroplasts from cells grown in the presence of 1 mM choline and enriched in phosphatidylcholine.

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are two of the major phospholipids synthesized by most strains of *Saccharomyces cerevisiae* (7, 18, 20). These phospholipids are synthesized in *S. cerevisiae* by both the cytidine nucleotide pathway and the methylation pathway (23). A proportionate increase in the synthesis of PC or PE can be induced in *S. cerevisiae* if a defined medium is supplemented with 1 mM choline (24, 25) or 1 mM ethanolamine (17). The present paper shows that isolated plasma membranes from *S. cerevisiae* NCYC 366 grown in a medium supplemented with choline or ethanolamine also contain increased proportions, respectively, of PC or PE, and that enrichment with PC predisposes spheroplasts from cells so enriched to osmotic lysis.

S. cerevisiae NCYC 366 was grown batchwise aerobically as already described (8, 21) except that the mixture of vitamins in the medium was replaced by 0.1% (wt/vol) yeast extract (Oxoid). Choline chloride and ethanolamine were sterilized by membrane filtration and included in the medium at 1 mM. Cells were harvested from midexponential-phase cultures (0.22 to 0.24 mg [dry-weight equivalence]) (8). Freshly harvested cells were converted into spheroplasts by digestion with a basidiomycete β-glucanase (9), and the volumes of the spheroplasts were measured as already described (6). Purified preparations of plasma membranes were obtained as already described after labeling the outer surface of spheroplasts by lactoperoxidase-catalyzed iodination (6, 12, 15). Lipids were extracted from cells by the method of Letters (11) with the modification already reported (6). Total phospholipid, individual phospholipids, triacylglycerols, and sterol esters were assayed as described by Hossack and Rose (6). Free sterols were separated according to Hunter and Rose (8) and assayed by the method of Moore and Baumann (14). Fatty acid methyl esters were analyzed by gas-liquid chromatography (8). The susceptibility of spheroplasts to osmotic lysis over the range of 0 to 1.2 M sorbitol was measured as described by Alterthum and Rose (1). All chemicals used were AnalaR or of the highest purity available commercially. Beta-glucanase from basidiomycete QM 806 was prepared as already reported (1).

Growth of cells in media supplemented with choline or ethanolamine led to an enrichment of the cell and plasma phospholipids with, respectively, PC and PE (Table 1). Although phosphatidylinositol and phosphatidylserine could not be separated completely on thin-layer chromatography plates, examination of plates under ultraviolet radiation showed that the former phospholipid always accounted for the bulk of the mixture. Plasma membranes from cells grown in the presence of either supplement contained a greater proportion of these two phospholipids compared with the total cell lipids. Plasma membranes were devoid of cardiolipin, although this phospholipid was detectable in total cell lipids. Cells grown in the presence of either supplement contained virtually the same levels of total phospholipid and of free sterols (Table 2) and had almost identical fatty acyl compositions. The molar ratios of phospholipid to sterol in plasma membranes from cells grown in each medium were in the range of 5:1 to 6:1. Whereas the triacylglycerol content of cells grown in the presence of each of the supplements was virtually the same, cells grown in the presence of ethanolamine synthesized greater quantities of sterol ester compared

TABLE 1. *Phospholipid composition of cells and plasma membranes of Saccharomyces cerevisiae NCYC 366 grown in medium supplemented with choline or ethanolamine*

Phospholipid	Percentage composition with cells grown in:			
	Choline-supplemented medium		Ethanolamine-supplemented medium	
	Cells	Membranes	Cells	Membranes
Phosphatidylcholine	46.2	32.5	35.0	25.7
Phosphatidylethanolamine	27.8	18.6	40.7	36.5
Phosphatidylinositol + phosphatidylserine	13.9	27.2	10.7	25.3
Cardiolipin	3.8	ND[a]	3.6	ND
Phosphatidic acid	3.8	18.3	4.7	6.9
Lysophospholipids	3.8	ND	4.4	ND
Base spot	0.9	3.5	0.9	5.7

[a] ND indicates that the phospholipid could not be detected.

TABLE 2. *Contents of major classes of lipid in Saccharomyces cerevisiae NCYC 366 grown in medium supplemented with choline or ethanolamine*

Lipid class	Content[a] (mg/100 mg [dry wt]) in cells grown in:	
	Choline-supplemented medium	Ethanolamine-supplemented medium
Phospholipids	3.42 ± 0.28 (3)	3.41 ± 0.24 (4)
Free sterols	0.22 ± 0.07 (5)	0.21 ± 0.07 (5)
Sterol esters	0.24 ± 0.03 (5)	0.42 ± 0.10 (5)
Triacylglycerols	0.53 ± 0.19 (4)	0.56 ± 0.18 (3)

[a] Values quoted are means ± 95% confidence limits. The number of independent determinations made is indicated in parentheses.

with those grown in choline-supplemented medium.

Cells grown in media containing either supplement were equally susceptible to digestion with β-glucanase. When spheroplasts were suspended in buffer containing hypotonic concentrations of sorbitol, there was a decrease in the absorbance of the suspension (Fig. 1); microscopic observation showed that this was caused largely by lysis of spheroplasts, although some decrease must have been attributable to spheroplasts swelling. When suspended in buffer containing 1.0 to 0.7 M sorbitol, spheroplasts with membranes enriched in PE were more resistant to osmotic lysis than those with membranes enriched in PC (Fig. 1). The average volume of spheroplasts that remained intact after dilution into hypotonic solutions of buffered sorbitol was greater with spheroplasts enriched in PC rather than PE (Fig. 2).

Since spheroplasts enriched in PE were more resistant to osmotic lysis than those enriched in PC, plasma membranes enriched in the former phospholipid must have a greater capacity to remain intact than those enriched in PC. It is worth noting that the difference in stability was much greater than the difference in phospholipid enrichment, which could indicate that the arrangement of PC and PE in the membrane is an important factor. Since the volume of surviving spheroplasts was greater when these were enriched with PC rather than PE, it might be concluded that the stability

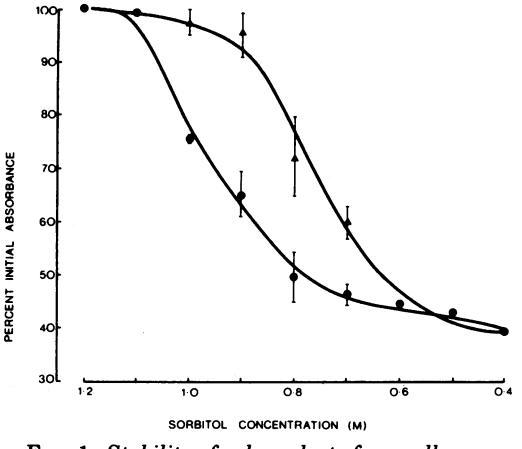

FIG. 1. *Stability of spheroplasts from cells grown in the presence of choline (●) or ethanolamine (▲) when suspended in hypotonic solutions of buffered sorbitol. Portions (0.1 ml) of spheroplast suspension (containing 10 mg (dry-weight equivalence) per ml of buffered sorbitol) were added to 2.9-ml portions of buffer containing concentrations of sorbitol ranging from 1.2 M to zero. The contents of each tube were then shaken gently and maintained at room temperature (18 to 22°C) for 10 min, and the absorbance ($E^{600}_{1\,cm}$) of the suspension measured. Each value plotted is the average of at least three independent observations. The vertical bars indicate 95% confidence limits on values.*

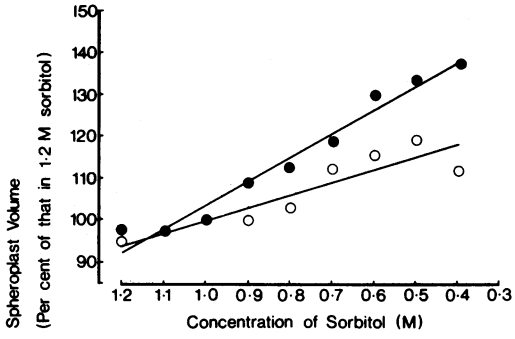

FIG. 2. *Percentage increase in average volumes of surviving spheroplasts prepared from cells grown in the presence of choline (●) or ethanolamine (○) after dilution into hypotonic solutions of buffered sorbitol containing 0.2 M sodium chloride. The lines of best fit were calculated by regression analysis.*

conferred on membranes enriched in PE results from their capacity to resist stretching. The larger size of the surviving spheroplasts enriched in PC could also be attributable, in part at least, to a higher internal osmolality and lower membrane permeability. While these possibilities cannot be discounted, it is unlikely that they are of major importance since differences in internal osmolality and membrane permeability are not likely to exist in cells grown at the same rate (17) in media very similar in composition.

Two observations from studies on artificial membranes are relevant when attempting to arrive at a molecular explanation of the effect of PC and PE on yeast membrane stability. Demel et al. (4) and Mikulecky and Tobias (13) reported data indicating that the bulkier zwitterion of PC compared with PE keeps the fatty acyl chains further apart, which in turn could lead to increased instability. In addition, Phillips and his colleagues (16) discovered from X-ray analysis of PC dispersions that the preferred orientation of the PC zwitterion is normal to the bilayer, whereas zwitterions of PE tend to lie tangentially to the bilayer. They concluded that these differences in orientation explain why the zwitterion of PC is more easily hydrated (16). Since hydration of the head groups must be an early event in osmotic lysis, a process that involves entrance of water molecules into the hydrophobic interior of the plasma membrane bilayer, the different orientations of the zwitterions could explain the differences observed in the stability of spheroplasts enriched in PC as compared with PE. The effect would be more pronounced if, as with erythrocyte membranes (2, 3, 26), PC molecules tend to be concentrated in the outer monolayer of the bilayer and PE in the inner monolayer.

The availability of a variety of mutants of *S. cerevisiae* that require either an unsaturated fatty acid (5, 19) or a sterol (10) for aerobic growth, and exploitation of the anaerobically induced requirements in this yeast for both of these classes of compound (1, 6), have provided useful systems for a study of the roles of fatty acyl unsaturation and sterol composition in the functioning of yeast membranes. The system described previously (17) and elaborated in this paper is the only one available for enriching membranes in eukaryotic microbes with PC or PE (17), and it provides a very useful model system for examining the role of phospholipid composition in the functioning of membranes in eukaryotic microorganisms, one which could well be exploited in studies on the significance of different PC-PE proportions in the membranes of animal cells (22).

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