Phospholipid Composition and the Effect of Sodium Dodecyl Sulphate on *Saccharomyces cerevisiae*

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Populations of *Saccharomyces cerevisiae* with plasma membranes enriched in phosphatidylethanolamine lost viability and released cations at a greater rate when suspended in buffered sodium dodecyl sulphate than did populations with membranes enriched in phosphatidylcholine. However, when suspended in buffered sorbitol (1.2 M) containing sodium dodecyl sulphate, sphaeroplasts from organisms with phosphatidylcholine-enriched membranes released cations faster than did sphaeroplasts with membranes enriched in phosphatidylethanolamine. Liposomes prepared in potassium chloride from mixtures of phospholipids from organisms enriched in phosphatidylcholine or phosphatidylethanolamine lost potassium ions at the same rate when suspensions were supplemented with sodium dodecyl sulphate. Organisms enriched in phosphatidylcholine or phosphatidylethanolamine did not differ in appearance in scanning electron micrographs, in electrophoretic mobilities over the pH range 2 to 9, in the ease with which they were converted into sphaeroplasts by β -glucanase, or in permeability to a range of polyethylene glycols. Walls from phosphatidylcholine- and phosphatidylethanolamine-enriched organisms had the same contents of β -glucans, α -mannan and protein.

INTRODUCTION

Feingold and his colleagues (HsuChen & Feingold, 1973; Feingold *et al.*, 1974) showed that liposomes prepared from phosphatidylethanolamine were extremely sensitive to the surface-active antibiotic polymyxin, while structures prepared from *N*-methyl-substituted analogues of phosphatidylethanolamine, including phosphatidylcholine, were insensitive to the antibiotic. The present paper shows how the phospholipid composition of the plasma membrane in *Saccharomyces cerevisiae* affects its sensitivity to the surface-active agent sodium dodecyl sulphate (SDS). The study exploits the discovery (Hossack *et al.*, 1977) that the proportions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the plasma membrane of *S. cerevisiae* can be increased by growing the yeast in defined media supplemented with choline or ethanolamine.

METHODS

Experimental cultures. *Saccharomyces cerevisiae* NCYC 366 was maintained on slopes of malt extract/yeast extract/glucose/peptone (MYGP) agar medium (Wickerham, 1951). It was grown aerobically at 30 °C as described by Hunter & Rose (1972) except that the mixture of vitamins was replaced by yeast extract (Oxoid; 0.1 %, w/v). Smaller (100 ml) cultures were grown at 30 °C in 250 ml conical flasks shaken at 250 rev. min⁻¹ in a Gallenkamp orbital shaker. Where indicated, the medium was supplemented with choline chloride or ethanolamine, each at 1 mM, as described by Hossack *et al.* (1977). Organisms were harvested from late-exponential phase cultures (0.22 to 0.24 mg dry wt ml⁻¹) by centrifuging at 4 °C for 1 min at 12250 g. Those from which lipids were to be extracted were washed twice with water at 4 °C, freeze-dried

and stored at -20°C over silica gel, while organisms that were to be converted into sphaeroplasts were washed three times in imidazole/HCl buffer (10 mM; pH 6.0) containing 10 mM-MgCl₂ and 1.2 M-sorbitol. Except where otherwise indicated, other batches of organisms were washed three times in 67 mM-KH₂PO₄ (pH 4.5).

Viability measurements. Changes in the viability of populations were measured by suspending washed organisms at 0.16 mg dry wt ml⁻¹ in 67 mM-KH₂PO₄ (pH 4.5; 100 ml), supplemented with SDS as indicated, and shaking at 250 rev. min⁻¹ in a 250 ml conical flask at 30 °C. Portions were removed at intervals, diluted appropriately with 67 mM-KH₂PO₄, and quadruplicate 0.1 ml portions of the diluted suspension were spread on MYGP agar plates. Plates were incubated at 30 °C for 2 d, and then the numbers of colonies were counted. Viabilities are expressed as a percentage of the viable count at zero time.

Preparation of sphaeroplasts. Sphaeroplasts were prepared as described by Cartledge & Rose (1973) except that Zymolyase-5000 was used at 0.4 mg per 6 mg dry wt organisms as a source of β -glucanase. Sphaeroplasts were twice washed gently in buffered 1.2 M-sorbitol (lacking MgCl₂), resuspended in the same buffer, and used immediately or stored at 4 °C.

Preparation of liposomes. Lipids were extracted from organisms and separated and assayed using the techniques of Hossack & Rose (1976). A portion of chloroform containing 37.5 μmol of a mixture of yeast phospholipids or commercial phospholipids was dried in a 50 ml flask on a Büchi rotary evaporator at 45 to 50 °C. The last traces of solvent were removed from the flask by flushing with nitrogen gas for 1 min. A portion (3 ml) of imidazole/HCl buffer (50 mM; pH 6.0) containing 150 mM-KCl was added to the flask, the head space was flushed with nitrogen gas, and the flask was gently turned by hand until all of the phospholipid was removed from the walls of the flask. Resuspension of phospholipid was aided by including two glass beads (2.0 mm diam.) in the flask. The suspension of liposomes was then subjected to ultrasound for 5 min, using a M.S.E. 100 W ultrasonicator, fitted with a 3 mm diam. probe, at maximum amplitude. The tube containing the liposome suspension was immersed in an ice/water mixture whilst in the ultrasonicator, and the head space was continually flushed with nitrogen gas. The ultrasonicated suspension, which contained liposomes of a fairly uniform dimension (1.0 to 1.5 μm diam.), was then dialysed in Visking tubing for 2 to 3 h against imidazole/HCl buffer containing 150 mM-MgSO₄, the buffer being changed three times during this period. Suspensions of liposomes were used immediately or stored at 4 °C.

Release of cations from organisms, sphaeroplasts and liposomes. This was measured using an EIL 7050 pH/selective ion meter fitted with an EIL K⁺ selective glass electrode (33 1057 200) and a sulphate reference electrode (33 1370 230) coupled to a potentiometric recorder (Smiths Servoscribe). The electrode was equally sensitive to K⁺ and NH₄⁺, and 25% as sensitive to Na⁺. Measurements were carried out in plastic tubes placed in a jacketed water bath maintained at 25 °C, and the contents were agitated by a magnetic stirrer. Organisms and sphaeroplasts were washed twice in imidazole/HCl buffer (10 mM; pH 6.0) containing 1.2 M-sorbitol, and resuspended in the same buffer. A portion of the suspension containing 5 mg dry wt equiv. of organisms or sphaeroplasts, or 1.0 μmol phospholipid equiv. of liposomes in imidazole/HCl (50 mM; pH 6.0) buffer containing 150 mM-MgSO₄, was placed in the plastic tube and, after the recorder base line had stabilized, the volume of suspension was made up to 10 ml with the appropriate buffer supplemented with SDS to give the required final concentration. The instrument was calibrated using standard solutions of KCl, and the release of ions from organisms, sphaeroplasts and liposomes expressed as the percentage released of the total content of K⁺ equivalents. The content of K⁺ equivalents in organisms was determined by placing a standard suspension (5 mg dry wt in 10 ml) in a bath of boiling water for 30 min, cooling, and measuring the content of K⁺ equivalents in the suspension after making up to 10 ml. Cations were released from sphaeroplasts and liposomes by incorporating Triton X-100 at 1.0 mM.

Preparation and analysis of walls. Walls were prepared as described by Jayatissa & Rose (1976) except that the pellet of walls and unbroken organisms was washed with 0.9% (w/v) NaCl three times or until the supernatant liquid became clear. In addition, the suspension of walls in water was placed in a bath of boiling water for 10 min before the organelles were washed 10 times with water. Contents of glucan and mannan in walls were determined as described by McMurrough & Rose (1967). Protein was extracted from walls (3 mg dry wt) in an acid-washed tube by adding 1.5 M-NaOH (0.2 ml) and 0.4 ml water, sealing the tube and steaming it for 15 min. Protein in the extract was assayed by the method of Lowry *et al.* (1951). The porosity of walls and plasma membranes in organisms was determined using a range of polyethylene glycols of finite number-average molecular weight as described by Scherrer *et al.* (1974).

Measurement of electrophoretic mobility. Measurements were made at different pH values as described by Jayatissa & Rose (1976), except that buffers were prepared by diluting stock solutions to an ionic strength of 0.05 and adjusting to the required pH value using HCl (ionic strength 0.05).

Scanning electron micrographs. Organisms were fixed in 50 mM-phosphate buffer (pH 7.0) containing 3% (w/v) glutaraldehyde for 1 h with occasional stirring. After centrifugation, they were dehydrated for 10 min in 50% (v/v) and then 80% (v/v) ethanol, and finally three times for 15 min in absolute ethanol. They

were then suspended in acetone and dried in a Polaron E 5000 critical-point drier. Dried cells were attached to a conductive stub with double-sided cellulose tape, coated with gold using a Polaron sputter coater, and examined in a Cambridge S 4 Stereoscan electron microscope.

Chemicals. All chemicals used were analytical reagent grade or of the highest purity available commercially. SDS (specially purified) was purchased from BDH. Zymolyase-5000 was obtained from the Kirin Brewery Co. Ltd, Takasaki, Gumma Pref., Japan. Polyethylene glycols of finite number-average molecular weight were purchased from Koch-Light, and tritiated water from The Radiochemical Centre, Amersham.

RESULTS

Effect of SDS on growth, viability and cation release

Growth of *S. cerevisiae* NCYC 366 was inhibited when as little as 0.01 mM-SDS was incorporated in the medium, although the rate of growth was severely retarded only when the SDS concentration was increased to 0.1 mM. Populations of organisms with plasma membranes enriched in PC remained viable to a greater extent when suspended in phosphate buffer (pH 4.5) containing 0.1 mM-SDS compared with organisms having membranes enriched in PE. There was no loss of viability in populations of organisms with either enrichment when suspended in SDS-free buffer (Fig. 1). After 2.5 min, the rate of release of cations from organisms enriched in PE and suspended in buffered 1.2 M-sorbitol containing 1.0 mM-SDS became progressively greater than the rate of release from PC-enriched organisms (Fig. 2). There was no detectable release of cations from organisms suspended in SDS-free buffer.

Release of cations from sphaeroplasts and liposomes by SDS

Sphaeroplasts with membranes enriched in PC released cations more rapidly when the suspension was supplemented with 1.0 mM-SDS compared with sphaeroplasts with membranes enriched in PE (Fig. 3). There was no detectable release of cations from sphaeroplasts suspended in SDS-free buffer.

In agreement with Papahadjopoulos & Miller (1967) and HsuChen & Feingold (1973), we were unable to form effectively sealed liposomes using pure PE. Liposomes prepared from a mixture of PC, PE and phosphatidic acid (86:10:4, by wt) released K^+ at the same rate as liposomes prepared from a mixture of PC and phosphatidic acid (96:4, w/w) when the suspension was supplemented with 0.5 mM-SDS. Liposomes prepared from mixtures of phospholipids extracted from organisms enriched in PC or PE leaked K^+ at the same rate when suspensions were supplemented with 0.50 or 0.75 mM-SDS (Fig. 4). Liposomes suspended in SDS-free buffer did not release detectable amounts of K^+ .

Properties of walls from organisms enriched in PC or PE

Scanning electron micrographs of organisms enriched in either phospholipid did not reveal differences in size, shape or surface appearance, while the electrophoretic mobilities of organisms, in the range pH 2.0 to 9.0, were the same irrespective of the nature of the phospholipid enrichment, which indicates that there were no differences in the nature of wall-surface ionogenic groups. Organisms enriched in PC or PE were converted into sphaeroplasts at approximately the same rate, suggesting that there were probably no major differences in the content of β -glucans in the walls or in the structure of the β -glucans. Analyses of walls confirmed that there were no differences in the contents of β -glucans or in the mannan and protein contents. Plots of percentage uptake of a range of polyethylene glycols against molecular weight gave virtually identical curves, with a monodisperse ethylene glycol of molecular weight approximately 145 corresponding to the exclusion value for penetration of the plasma membrane, and a polydisperse polyethylene glycol of number-average molecular weight approximately 650 corresponding to the exclusion value for penetration of the wall. The molecular weight of SDS is 288.

Fig. 1

Fig. 2

Fig. 1. Viability of populations of *Saccharomyces cerevisiae* NCYC 366 grown in the presence of choline (●) or ethanolamine (○), and suspended in phosphate buffer (pH 4.5) either lacking (---) or supplemented (—) with SDS (0.1 mM). The 95% confidence limit on the curve describing viability in PC-enriched populations is $\pm 7.6\%$, and in PE-enriched populations $\pm 5.36\%$. Values are means of four results from each of two independent determinations.

Fig. 2. Time-course of release of K^+ equivalents from *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in PC (●) or PE (○) suspended in buffer (pH 6.0) containing 1.2 M-sorbitol and 1.0 mM-SDS. The 95% confidence limit on the curve describing release from PC-enriched organisms is $\pm 0.78\%$, and from PE-enriched organisms $\pm 3.51\%$. Values plotted are the average of three determinations.

Fig. 3

Fig. 4

Fig. 3. Time-course of release of K^+ equivalents from sphaeroplasts of *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in PC (●) or PE (○) suspended in buffer (pH 6.0) containing 1.2 M-sorbitol and 1.0 mM-SDS. The 95% confidence limit on the curve describing release from PC-enriched sphaeroplasts is $\pm 3.63\%$, and from PE-enriched sphaeroplasts $\pm 3.12\%$. Values plotted are the average of three determinations.

Fig. 4. Time-course of SDS-induced release of K^+ from liposomes prepared from mixtures of phospholipids extracted from *Saccharomyces cerevisiae* NCYC 366 enriched in PC (●) or PE (○): suspensions were supplemented with SDS at 0.75 mM (—) or 0.50 mM (---). Values plotted are the average of three determinations.

DISCUSSION

The ability of SDS to penetrate yeast plasma membranes enriched in PE more easily than those enriched in PC is most likely explained by the preferred orientations of the polar headgroups in the outer monolayer of the membrane. The ethanolamine headgroup is believed to be oriented parallel to the surface of membranes (Hitchcock *et al.*, 1974; Akutsu *et al.*, 1975; Seelig & Gally, 1976). A study of X-ray long spacing of dispersions of PC led Phillips *et al.* (1972) to conclude that the choline headgroup lies perpendicular to the membrane. Although it has recently been contested (Seelig *et al.*, 1977), such an orientation would make it more susceptible to hydration than the ethanolamine headgroup and render PE-enriched membranes more susceptible to penetration by the hydrophobic chain of SDS. In support of this view is the finding of Jendrasiak & Hasty (1974) that films of PC have higher affinity for water than do PE films.

It is reasonable to assume that phospholipids in the bilayers of liposomes were more or less randomly distributed both in the planes of the monolayers and between the outer and inner monolayers. Since liposomes prepared from phospholipids from PC- and PE-enriched organisms were equally susceptible to SDS-induced release of K^+ , the differences in the effect of SDS on organisms enriched in each phospholipid are probably caused by a non-random distribution of phospholipids in the plasma membrane. One established type of non-random distribution of phospholipids in plasma membranes is asymmetric distribution of molecules between each of the bilayers. Among micro-organisms, asymmetry has so far been demonstrated only in bacteria, which differ in that some have PE concentrated in the outer monolayer (Bishop *et al.*, 1977; Paton *et al.*, 1978) whereas others concentrate this phospholipid in the inner monolayer (Rothman & Kennedy, 1977). If, as seems conceivable, there is asymmetric distribution of phospholipids in the plasma membrane of *S. cerevisiae*, the greater sensitivity of PE-enriched organisms to SDS could be due to a concentration of this phospholipid in the outer monolayer.

Differences in the sensitivity to SDS between organisms and sphaeroplasts, enriched in PE or PC, might be explained by another type of non-random distribution of phospholipids in the membrane, namely one in the plane of the membrane. This type of non-random distribution, a type of mosaicism, has yet to be demonstrated, but its existence can be inferred from the furrowed appearance of the outer surface of sphaeroplasts from *S. cerevisiae* (Takeo *et al.*, 1976). Increased susceptibility to SDS in organisms with membranes enriched in PE might be a result of the existence in these membranes of PE slicks which, when of sufficient size, could constitute areas that are particularly susceptible to SDS penetration. The existence of such slicks would be compatible with the data reported by Jendrasiak & Mendible (1976) on hydration of PC/PE mixtures. Following removal of the wall during sphaeroplast formation, these slicks, which are possibly fixed in position by membrane components that are bound to the inside of the wall, would be dispersed, giving rise to a distribution of PC and PE in the outer monolayer that renders it differently, and seemingly oppositely, susceptible to SDS penetration.

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