

Preparation of phosphatidyl inositol from bakers' yeast

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SUMMARY Phosphatidyl inositol and a cardiolipin-like phospholipid survive the autolysis which is induced in yeast by treating it with toluene, whereas other phospholipids are extensively degraded. Lipid from autolyzed yeast was suspended in isopropanol to extract lecithin and neutral lipid. The insoluble phosphatidyl inositol and cardiolipin were then separated by chromatography on silicic acid.

KEY WORDS yeast · autolysis · toluene · phosphatidyl inositol · cardiolipin

DETERMINATION OF phospholipids by chromatography on silicic acid-impregnated paper shows that commercial bakers' yeast (Distillers Company Ltd.) contains about 11 mg/g dry weight of phosphatidyl inositol, or 22% of the total phospholipid.¹ The preparation of this phospholipid from yeast is not, however, entirely straightforward.

¹ Analytical data on yeast lipids, being prepared for publication.

Because of the limited porosity of the cell wall, phospholipids are not extracted quantitatively from unbroken yeast cells (1). Other phospholipids present in yeast extracts may interfere with the separation of phosphatidyl inositol by chromatography on silicic acid (2).

Both these difficulties are partially overcome by first autolyzing yeast for 5 hr at 38°C in the presence of toluene. Lipid is now readily extracted by chloroform-methanol. The ratio of phosphatidyl inositol to other phospholipid is increased, since during autolysis enzymes are activated which completely degrade the phosphatidyl serine of the yeast, and cause extensive loss of lecithin and of phosphatidyl ethanolamine (1), with the formation of free fatty acid.

Apart from the phospholipids mentioned, commercial yeast contains about 3 mg/g of an acidic phospholipid which resembles cardiolipin (3) in giving a fast-running, streaky spot when it is chromatographed on paper in diisobutyl ketone-acetic acid-water.¹ This phospholipid also survives autolysis.

Yeast cardiolipin and phosphatidyl inositol were found to be sparingly soluble in lower alcohols, and this property enabled a considerable degree of purification to be effected. The two phospholipids are easily resolved on a column of silicic acid.

Autolysis and Extraction. Bakers' yeast was broken into small pieces by forcing it through stainless steel gauze. To 100 g fresh weight of it (29 g dry weight), in a liter flask, 10 ml of toluene was added. The flask was loosely sealed with aluminum foil and incubated for 5 hr at 38°C. Lipid was extracted by stirring the yeast overnight at 30°C, under nitrogen, with 700 ml of chloroform-methanol 1:1 (v/v). The filtered extract was washed with 240 ml of 0.2 M NaCl. The yeast residue was extracted for 1 hr at room temperature with 300 ml of chloroform-methanol 2:1 (v/v), this extract being washed with 75 ml of 0.2 M NaCl. The combined lower phases were evaporated in a rotary film evaporator, benzene-ethanol 8:3 (v/v) being added toward the end to assist in the removal of toluene and water. The residual lipid was a dark yellow semisolid.

Removal of Lecithin and Neutral Lipid. The lipid was shaken with isopropanol (50 ml/100 g yeast), and left overnight at room temperature. The white, granular precipitate which settled out was collected by filtration through sintered glass, washed with small quantities of isopropanol, and dissolved in chloroform.

Silicic Acid Chromatography. The solution of isopropanol-insoluble lipid was applied to a 29 × 2.5 cm column made from a slurry in chloroform of 25 g of Whatman SG. 34 silicic acid (H. Reeve Angel & Co. Ltd., London, England), which had been dried at 110°C overnight. This corresponded to a column loading of 12 mg of phosphatidyl inositol per gram of adsorbent. (With

24 mg/g the phosphatidyl inositol preparation was found to be contaminated with traces of cardiolipin.) The column was then washed with 200 ml of chloroform to remove neutral lipid.

Chloroform-methanol 4:1, 125 ml, eluted most of the cardiolipin. A further 125 ml eluted the rest, together with phosphatidyl ethanolamine, this fraction being discarded. Finally, phosphatidyl inositol was eluted with 255 ml of chloroform-methanol-water 25:25:1.

Phosphatidyl Inositol. The chloroform-methanol-water eluate was evaporated to give crude phosphatidyl inositol in yields of about 10 mg/g of yeast, and this was dissolved in chloroform-methanol 2:1. The solution was washed with 0.25 volume of 0.1 M citrate buffer (2 moles Na salt: 1 mole acid, pH 5.2) to remove heavy metals which might otherwise promote autoxidation and to form the sodium salt of the phospholipid. Citrate was washed out of the lower phase with chloroform-methanol-water 3:48:47 (4). The phospholipid solution was evaporated to give a white, granular solid, which was deposited in lumps.

For getting the material in a form suitable for collection, the procedure of Gray and Macfarlane (5) was found to be greatly superior to precipitation with acetone (2). About 600 mg of phosphatidyl inositol was dissolved in the nonpolar solvent chloroform, which was then evaporated to give a yellowish wax. Unlike the white granular material recovered from polar solvents, this dissolved instantly in 25 ml of ether, presumably because the nonpolar groups of the solid phosphatidyl inositol were directed outward. Methanol (10 ml) was added dropwise, and the preparation was allowed to stand at -5°C for 3 days. The phosphatidyl inositol was collected by filtration and dried in vacuo over silica gel. Yield was 9.9 mg/g of yeast, dry weight. Solid and solutions were colorless.

Chromatograms on Whatman SG.81 silicic acid-impregnated paper developed with diisobutyl ketone-acetic acid-water 80:40:7 or 8:5:1 showed a spot with R_f 0.1-0.2, depending on the water content of the solvent and on other conditions. A trace of phosphatidyl ethanolamine was the only ninhydrin-positive component. Lecithin was not detected. Cardiolipin was present in traces only when the recommended column loading had been exceeded. Chromatograms were also developed by the descending technique for 3 days in chloroform-acetic acid-water 40:25:4, when the phospholipid migrated a distance of 34 cm. Only a faint trace of some slower-running component was detected.

Higher inositides were sought by chromatographing the phospholipid on formaldehyde-treated Whatman 3MM paper, prepared and developed according to the directions of Hendrickson and Ballou (6). None were

found. Phosphatidyl inositol had an R_f of 0.46 in this system.

Yeast phosphatidyl inositol was deacylated at room temperature with 0.1 M KOH in chloroform-methanol 2:3, and the water-soluble products examined by paper chromatography. Glycerolphosphoryl inositol, with a trace of glycerolphosphoryl ethanolamine, was identified.

Fatty acid methyl esters were prepared by refluxing a solution of the phospholipid in benzene with methanolic HCl (7). The Perkin-Elmer Model 800 instrument used for gas chromatography was fitted with a flame-ionization detector. The $1/8$ inch o.d. stainless steel column was 2 m long, and was packed with butanediol succinate polyester, 8% on silanized Chromosorb W, 80-100 mesh. Column temperature was 180°C, nitrogen flow 30 ml/min. The predominant components of two preparations examined were methyl palmitate (25.6 and 21.1%), palmitoleate (30.9 and 31.8%), stearate (8.6 and 7.4%), and oleate (31.5 and 35.4%). Phosphatidyl inositol was therefore relatively rich in the saturated fatty acids, the corresponding percentages for the mixed phospholipids from this type of yeast being respectively 11.9, 48.8, 3.3, and 29.3.¹ The molar ratios of fatty acid:phosphorus were 1.9 and 2.0.

The methanol-water phase left after methanolysis of yeast phosphatidyl inositol and extraction of fatty acid methyl esters was evaporated to a small volume and heated in a sealed tube with 6 N HCl for 48 hr at 105°C. The hydrolysate was chilled, and kept for 4 days in vacuo over solid NaOH, when a white, crystalline solid remained. This was identified as inositol by paper chromatography (8) and by gas-liquid chromatography of its trimethylsilyl ether (9). Inositol eluted from paper chromatograms was determined from the periodate consumed after reaction for 18 hr at room temperature. The inositol content of a preparation of yeast phosphatidyl inositol corresponded to a molar ratio, inositol:phosphorus, of 1.0.

The phosphorus contents of three preparations were 3.57, 3.52, and 3.55%. After being dried over P_2O_5 , yeast phosphatidyl inositol contained² C 58.9, H 9.6, P 3.61, N 0.20, Na 2.42, K 0.04, Mg 0.015%. Calculated for the sodium salt of phosphatidyl inositol having the fatty acid composition stated above, C 59.9, H 9.3, P 3.64, N nil, Na 2.70. The nitrogenous impurity may be protein, which separates as an interfacial film when hydrolysates are extracted with petroleum ether.

Yeast Cardiolipin. Lipid recovered from the chloroform-methanol 4:1 eluate was rechromatographed (5) on a small column of silicic acid to yield a phospholipid containing 3.60% phosphorus. It gave a single spot when it was chromatographed on silicic acid-impregnated

paper. The deacylated derivative also showed as a single spot on paper, with an R_f value relative to glycerol 3-phosphate of 2.0 in isopropanol-ammonia, as reported for the deacylation product of bovine heart cardiolipin (10). The presence of diphosphatidyl glycerol (cardiolipin) in yeast has been reported by several authors (11, 12), though no method of preparation has been described.

Palmitoleic and oleic acids, in approximately 1:1 molar ratio, accounted for nearly all the fatty acids (93%) of yeast cardiolipin.

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