981. *The Structure of a Lysophosphatidylcholine.*

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The lysophosphatidylcholine isolated from *Saccharomyces cerevisiae* has been shown to be 3-*O*-acyl-D-glycerol 1-(choline phosphate).

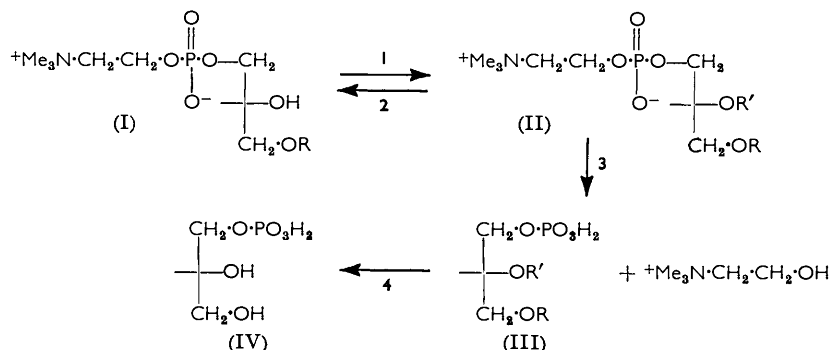
SMALL quantities of lysophosphatidylcholine have been reported in lipid extracts of mammalian tissues [1] and of bacteria.[2] In the present investigation a lysophosphatidylcholine has been isolated from whole cells of *Saccharomyces cerevisiae* grown aerobically for 51 hr. The lipids were extracted with aqueous acetone and chloroform–methanol, and the pure lyso-compound was isolated by chromatography on alumina and on silicic acid. The lipid was a colourless wax with analytical figures corresponding to those of a lysophosphatidylcholine. It is below shown to have structure (I; R = acyl), in which the stereochemistry is defined. The fatty acids were not characterised.

The lipid was hydrolysed under mildly alkaline conditions [3] to glycerol choline phosphate (I; R = H), that was further hydrolysed (by N-sodium hydroxide) to choline and glycerol 1- and 2-phosphate.

Since the stereochemistry of the glycerol phosphate residue, and the position of esterification of the fatty acid on glycerol, have not been reported for the lysophosphatidylcholines isolated by previous workers, we have determined these structural features in yeast lysophophatidylcholine. The lyso-compound was converted into a synthetic phosphatidylcholine (II; R = acyl, R′ = myristoyl) by acylating its cadmium chloride complex with myristoyl chloride.[4] The synthetic lipid was completely degraded by snake-venom phospholipase A to a lysophosphatidylcholine and free fatty acids. Gas chromatography showed that more than 90% of the fatty acids released was myristic acid

[1] Webster and Thompson, *Biochim. Biophys. Acta*, 1962, **63**, 38.
[2] Dawson, *Biochim. Biophys. Acta*, 1954, **14**, 374.
[3] Brown, Hall, and Letters, *J.*, 1959, 3547.
[4] Hanahan, Brockerhoff, and Barron, *J. Biol. Chem.*, 1960, **235**, 1917.

(see Table). Since phospholipase A is known to be specific for the release of fatty acids attached to the glycerol 2-position in phosphatidylcholines,[5] the above results show that the original lysophosphatidylcholine was esterified on the glycerol 1-position.



Reagents: 1, Myristoyl chloride, $CdCl_2$, pyridine. 2, Phospholipase A. 3, Phospholipase D. 4, NaOMe.

The synthetic phosphatidylcholine and yeast phosphatidylcholine (isolated from yeast after 48 hr. growth) were completely degraded to lyso-compounds by snake venom. Since this enzyme has been shown [6] to act only on lecithins in which the glycerol phosphate residue has the D-1 configuration it follows that the original lyso-compound has the same stereochemistry as yeast phosphatidylcholine. Further, degradation of the synthetic phospholipid (II; R = acyl, R′ = myristoyl) with cabbage-leaf phospholipase D gave a phosphatidic acid (III), which was further degraded to a glycerol phosphate (IV) by mild alkali. The glycerol phosphate (IV) was shown to be the *D*-1 isomer by a method described previously.[7]

The occurrence of lyso-compounds in lipid extracts is usually assumed to be due to the action of phospholipase A on the parent phospholipid, or to the hydrolysis of enol-ether linkages in the plasmalogen forms of these parent phospholipids. The latter explanation is not applicable in the present case as yeast phosphatidylcholine is completely converted into water-soluble phosphate compounds under mildly alkaline conditions.[3] However, since the disappearance of phosphatidylcholine in lipid extracts from yeast coincides with the appearance of a corresponding lyso-compound with the same stereochemistry, it seems reasonable to conclude that the latter is produced by the action of a phospholipase A on phosphatidylcholine.

Experimental

Paper Chromatography.—Phospholipids were chromatographed by the ascending technique for 14 hr. on formaldehyde-treated [8] Whatman No. 1 paper with the butan-1-ol–water–acetic acid. Chromatograms were dipped in Rhodamine B solution and inspected under an ultra-violet source to locate lipid spots. Choline lipids were detected with the Dragendorff reagent.[9] R_F values were: phosphatidylcholine 0·80; lysophosphatidylcholine 0·53.

Descending-front chromatography of water-soluble phosphate esters was carried out on Whatman No. 1 paper with a phenol–acetic acid solvent.[2]

Growth of Organism.—*Saccharomyces cerevisiae* (Guinness strain 1164) was grown in batches (6 l.) under forced oxygenation for 51 hr. at 21° in a medium containing: $(NH_4)_2SO_4$, 2 g.; KH_2PO_4, 2 g.; $CaCl_2$, 0·3 g.; $MgSO_4,7H_2O$, 0·5 g.; peptone, 5 g.; yeast extract 5 g.; glucose, 50 g.; water, 1 l. The organisms were harvested by centrifugation at 4° (yield, 7—8 g. wet wt. of organism per l. of culture).

[5] de Haas and van Deenen, *Biochem. Biophys. Res. Comm.*, 1960, **3**, 287.
[6] Long and Penny, *Biochem. J.*, 1957, **65**, 382.
[7] Brown, Clark, and Letters, *J.*, 1961, 3774.
[8] Hörhammer, Wagner, and Richter, *Biochem. Z.*, 1959, **331**, 155.
[9] Wagner, Hörhammer, and Wolff, *Biochem. Z.*, 1961, **334**, 175.

Extraction of Lipids.—Yeast (510 g. wet wt.) was extracted with acetone (1·5 l.) overnight at room temperature with continuous stirring and then for 2 hr. with 2 : 1 v/v chloroform–methanol (1·5 l.). The acetone extract was evaporated to dryness and the residue was dissolved in the chloroform–methanol extract which was washed with water (150 ml.). The organic phase was evaporated to dryness. The residue (4·8 g., 3·5% of dry wt., equiv. to 17·3 mg. of P) was dissolved in chloroform (100 ml.) and stored at 4°.

Isolation of Lysophosphatidylcholine.—A lipid sample (equiv. to 16 mg. of P) in chloroform (90 ml.) was applied to a column (38 × 1·2 cm.) of alumina (Merck, standardised according to Brockmann). The lipids were washed on with chloroform (300 ml.), and elution was with 1 : 1 v/v chloroform–methanol (10 ml./min.). Fractions were collected (25 ml.) and their lipid components analysed by paper chromatography. Phosphatidylcholine (equiv. to 0·8 mg. of P) was eluted in fractions 8—12. Lysophosphatidylcholine (equiv. to 9·3 mg. of P) was eluted in fractions 15—30 which were combined, evaporated to dryness, and applied (in 20 ml. of chloroform) to a column (12 × 1·2 cm.) of silicic acid. The column was eluted with chloroform–methanol (200 ml.) and then with methanol (1·5 l.). The methanol eluate was evaporated, to give a colourless residue which was dissolved in chloroform (1 ml.). A large excess of acetone was added to precipitate the lysophosphatidylcholine (97 mg.; overall, 0·07% of dry wt. of yeast) (Found, in material dried at 100° for 12 hr. *in vacuo* over P_2O_5: C, 59·0; H, 10·9; N, 2·9; P, 6·1. Calc. for $C_{24}H_{50}NO_7P$: C, 58·2; H, 10·1; N, 2·8; P, 6·25%). [The ratios glycerol :[10] phosphate : choline :[11] fatty acid ester (calc. as C_{16}) were 1·0 : 1·07 : 1·1 : 0·96].

D-*Glycerol* 1-(*Choline Phosphate*).—Lysophosphatidylcholine (62 mg.) was dissolved in 2 : 1 chloroform–methanol (4 ml.), and the solution was adjusted to pH 11—12 with methanolic sodium hydroxide and incubated at 37° for 20 min. Water (20 ml.) was added and then sufficient IRC-50(H^+) ion-exchange resin to adjust the pH to 5—6. The resin was filtered off and washed with water (2 × 10 ml.), and the filtrate and washings were centrifuged. The upper phase was applied to a column (10 × 1·2 cm.) of DEAE-cellulose (HCO_3^-) powder which was washed with water until the eluate was free from phosphate. The eluate was concentrated to 5 ml. and passed through a column (4 × 1 cm.) of Dowex-50(H^+) to remove traces of free choline, and the eluate from this column was freeze-dried, giving a white hygroscopic residue of D-glycerol 1-(choline phosphate) (23 mg.) (Found: N, 5·2; P, 11·9. Calc. for $C_8H_{20}NO_6P$: N, 5·4; P, 12·1%). The ratio glycerol : phosphate was 1 : 0·94. This phosphate diester (2 mg.) was heated at 60° for 30 min. with N-sodium hydroxide. Paper chromatography [3] of this hydrolysate showed the presence of glycerol 1- and 2-phosphate and choline.

Preparation of Synthetic Phosphatidylcholine.—Lysophosphatidylcholine (100 mg.) was converted into the cadmium chloride complex, which was acylated with myristoyl chloride according to the procedure of Hanahan *et al.*[4] The synthetic phosphatidylcholine (82 mg., 58%) was isolated by chromatography on silicic acid.[4]

Enzymic Degradation of Synthetic Phosphatidylcholine.—(*a*) *Phospholipase A*. The phosphatide (20 mg.) was dissolved in ether (10 ml.) and treated with an aqueous solution (0·2 ml.) of *Crotalus adamanteus* venom (3 mg.). The mixture was shaken vigorously for 2 min. and then set aside at room temperature for 2 hr. The gel which was formed was centrifuged off and washed with ether (2 × 5 ml.), and the residue was examined by paper chromatography. No phosphatidylcholine was detected in the residue, or in the supernatant liquid which was then subjected to chromatography on silicic acid. The fatty acid fraction was analysed on a Pye argon gas chromatograph with a stationary phase of diethylene glycol succinate and phosphoric acid.[12] The results are recorded in the Table, together with results obtained by analysis of the fatty acid fraction from alkaline hydrolysates of yeast phosphatidylcholine and lysophosphatidylcholine.

Composition of fatty acid fractions.

Fraction	C_{14} (%)	C_{16} (%)	C_{18} (%)
Yeast phosphatidylcholine	0	43	57
Yeast lysophosphatidylcholine	0	46	54
Fatty acids freed *	92	4	4

* Fatty acids freed by the action of snake venom on synthetic phosphatidylcholines.

[10] Hanahan and Olley, *J. Biol. Chem.*, 1958, **231**, 813.
[11] Wheeldon and Collins, *Biochem. J.*, 1958, **70**, 43.
[12] Jowett and Horrocks, *Nature*, 1961, **192**, 966.

(*b*) *Phospholipase D*. 0·1M-Ammonium acetate buffer (pH 5·7; 3 ml.), M-calcium chloride (0·5 ml.), and cabbage-leaf phospholipase D (10 mg.) were added to a solution of the phospholipid (60 mg.) in ether (3 ml.). The mixture was shaken at 25° for 2 hr., and the ether phase was separated. The aqueous phase contained 67% of the total choline. The ether layer was adjusted to pH 11—12 with methanolic sodium hydroxide and incubated at 37° for 20 min. Water (10 ml.) was added and then IRC-50(H^+) resin to adjust the pH to 5—6. The aqueous phase was shaken successively with light petroleum (5 ml.) and isobutyl alcohol (2 × 5 ml.). The aqueous phase, containing glycerol choline phosphate and glycerol 1-phosphate, was subjected to preparative paper chromatography and the glycerol 1-phosphate was obtained as its biscyclohexylammonium salt (7·8 mg.).[7] This salt was shown to be the D-1-isomer by enzymic and chemical mthods described previously.[7]

Isolation of Yeast Phosphatidylcholine.—Yeast was harvested after 48 hours' growth and the lipids were isolated as above (4·8% of dry wt. of yeast). These lipids (1·6 g.) were chromatographed on alumina in the same way as described for the isolation of lysophosphatidylcholine and similar chromatographic behaviour was obtained. The phosphatidylcholine fractions were combined (equiv. to 16 mg. of P) and the phospholipid was obtained as a viscous residue (380 mg.) on evaporation of the solvents. The lyso-compound, which was not isolated, was present in an amount equivalent to 1·2 mg. of phosphorus. The phosphatidylcholine (25 mg.) was hydrolysed to glycerol choline phosphate (I; R = H) in the same conditions as were used for the mild alkaline hydrolysis of the lyso-compound. The yield of water-soluble phosphate was 94% of the total phosphorus content, and no phospholipid was detected in the hydrolysate by paper chromatography. The phosphatidylcholine was degraded by snake-venom phospholipase A, with the system described above. Paper chromatography showed that conversion into a lysophosphatidylcholine was complete after 2 hr.

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