Phosphatidylglycerylglucosamine

By P. J. R. PHIZACKERLEY, J. C. MACDOUGALL and M. J. O. FRANCIS
Department of Clinical Biochemistry, University of Oxford

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In recent years it has become apparent that bacteria exhibit a considerably greater diversity in their phospholipid composition than do animal cells, and that several lipids, apparently unknown in animal tissues, may occur in bacteria (for review see Kates, 1964). The purpose of the present communication is to present evidence for the occurrence of a glucosamine derivative of phosphatidylglycerol in the cell-wall membrane of *Pseudomonas ovalis* Chester. A brief report by Op Den Kamp, Houtsmuller & Van Deenan (1965) suggests that a similar lipid may be present in *Bacillus megaterium*.

Methods. *Ps. ovalis* Chester was grown in liquid medium, and particles were prepared from ultrasonic extracts of the cells as described by Francis, Hughes, Kornberg & Phizackerley (1963). Lipids were extracted from the particles by the method of Folch, Lees & Sloane-Stanley (1957). Orthophosphate was estimated by the method of Bartlett (1959); total nitrogen was estimated by Kjeldahl ashing followed by ammonia estimation by the method of Fenton (1962); fatty acids were estimated by the method of Duncombe (1963), with palmitic acid as a standard; glycerol was estimated by the method of Wieland (1963) and L-3-glycerophosphate was estimated by the method of Hohorst (1963). The Elson-Morgan reaction for the estimation of 2-deoxyamino sugars was carried out by the method of Boas (1953) and ninhydrin degradation of these sugars by the method of Stoffyn & Jeanloz (1954).

Results and discussion. It has been shown previously that particles prepared as described above from ultrasonic extracts of *Ps. ovalis* Chester consist of fragments of the cell-wall membrane (Francis *et al.* 1963). These particles contain 110mg. of lipid and 4.7mg. of phospholipid P/g. particle dry wt. Examination of the lipid extracted from the particles by thin-layer chromatography on plates of silica gel G with the solvent system chloroform-methanol-water (65:25:4, by vol.) showed that at least seven phospholipids were present. This chromatogram was indistinguishable from that of a lipid extract obtained from cell-wall membranes prepared from this organism by the method of Hughes (1951). The ninhydrin-negative phospholipids together comprise 24% of the total, and, in addition to a small amount (0.8%) of phospholipid that remained at the origin, three

ninhydrin-positive phospholipids were detected. One of these is phosphatidylethanolamine (R_F 0.5) and it comprises 65% of the total phospholipid; another is lysophosphatidylethanolamine (R_F 0.3), which is present in small quantity (1–2%). The remaining ninhydrin-positive phospholipid (R_F 0.4) could not be identified with any known phospholipid. It accounts for 5.8% of the total phospholipid P and its reaction with ninhydrin is unusual. Thus when the plates were developed with 0.2% ninhydrin in butan-1-ol it yielded a pink derivative; when the ninhydrin spray was acidified with 2% (v/v) acetic acid it yielded a purple derivative; and when the ninhydrin spray was made alkaline with 2% pyridine it yielded a yellow derivative.

The unknown phospholipid was isolated by fractionating the lipid extract prepared from the particles by column chromatography on silicic acid. The column was eluted with chloroform, and then with mixtures of chloroform and methanol in the following proportions (v/v): 20:1, 9:1, 4:1, 3:2 and 1:4. The unknown lipid was eluted, together with some phosphatidylethanolamine, in chloroform-methanol (4:1, v/v). It was obtained as a single compound by preparative thin-layer chromatography. On analysis it was found to contain P, glycerol, fatty acid and total nitrogen in the proportions 1.0:1.8:1.8:1.0. These values are referred to P as unity, and the value for the glycerol content has been corrected for the 6% destruction of glycerol that occurs under the hydrolytic conditions employed (2N-HCl at 120° for 36 hr.). The value for the content of fatty acids would be expected to be about 10% too low when estimated by the method employed against a standard of palmitic acid (Duncombe, 1963). When subjected to strong acid hydrolysis (5.8N-HCl at 105° for 18 hr.), about 80% of the total P was released as orthophosphate and a single ninhydrin-positive compound was obtained. The following evidence indicates that this compound is glucosamine. First, it behaved as a reducing sugar and gave a positive Elson-Morgan reaction; secondly, it was found to have an R_F identical with that of glucosamine on paper chromatography in butan-1-ol-acetic acid-water (12:3:5, by vol.), phenol saturated with water, and phenol saturated with water-aq. ammonia (sp.gr. 0.88) (200:1, v/v). These systems, however, do not differentiate

between glucosamine and galactosamine; this distinction was made by chromatography in the solvent system pyridine-ethyl acetate-water-acetic acid (5:5:3:1, by vol.) (Fischer & Nebel, 1955), and by column chromatography by the method of Moore & Stein (1951) with a Technicon amino acid autoanalyser. In both cases the unknown material behaved as glucosamine, and was clearly differentiated from galactosamine. Finally, it was shown that ninhydrin degradation yielded arabinose, which is the product expected from glucosamine. Quantitatively, 74% of the expected amount of glucosamine was recovered after hydrolysis as described above, but since it was found in control experiments that the loss of glucosamine under these conditions averaged 24% it was concluded that the lipid under investigation contained 1 mole of glucosamine/g. atom of lipid P.

Further information about the structure of the lipid was obtained by examination of the products of partial acid hydrolysis (2.0N-HCl at 105° for 8 hr.). Under these conditions only 20% of the total P was liberated as orthophosphate, and the organic phosphates were found to be a mixture, in approximately equimolar proportions, of L- and D-3-glycerophosphate, which together account for about 70% of the total. In contrast with the results obtained on complete acid hydrolysis, two ninhydrin-positive compounds were formed, and these were well separated from each other, and from the phosphates, by high-voltage paper electrophoresis at pH 3.6 in pyridine-acetic acid-water (1:10:89, by vol.) at 100 v/cm. for 45 min. One of these

ninhydrin-positive compounds is glucosamine; the other, after hydrolysis in 5.8N-HCl at 105° for 18 hr., yields glycerol and glucosamine in equimolar proportions, and it is concluded therefore that this compound is glycerylglucosamine.

The results given above indicate that the lipid under discussion is a glucosamine derivative of phosphatidylglycerol, and suggest that the glucosamine moiety is covalently bound to a glycerol residue.

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The Morphological Origin and Function of the Polysomes Isolated from Rat Liver

By P. N. CAMPBELL, ELIZABETH LOWE and M. D. BUCKLEY SHARP

Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W. 1

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Wettstein, Staehelin & Noll (1963) were the first to report that aggregates of ribosomes, named by them 'ergosomes', but referred to here as 'polysomes', could be isolated from a rat-liver homogenate. They achieved this by treating a mitochondria-free supernatant with deoxycholate, centrifuging this over a cushion of dense sucrose (2.0M) and collecting the polysomes as a pellet. They showed in subsequent papers (Noll, Staehelin & Wettstein, 1963; Villa-Trevino, Farber, Staehelin, Wettstein & Noll, 1964) that the polysomes were

the active units in protein synthesis, and that the single ribosomes were of very low activity.

Bloemendal, Bont & Benedetti (1964) described the isolation of polysomes from liver without the use of deoxycholate and concluded that rat liver 'contains polysomal structures which are either not bound to the membranes of the endoplasmic reticulum or linked to the membranes by forces weaker than centrifugal forces'. Webb, Blobel & Potter (1964) compared the size distribution of polysomes isolated with and without deoxycholate,