

## The Structure of 'Phosphatidylglucose'

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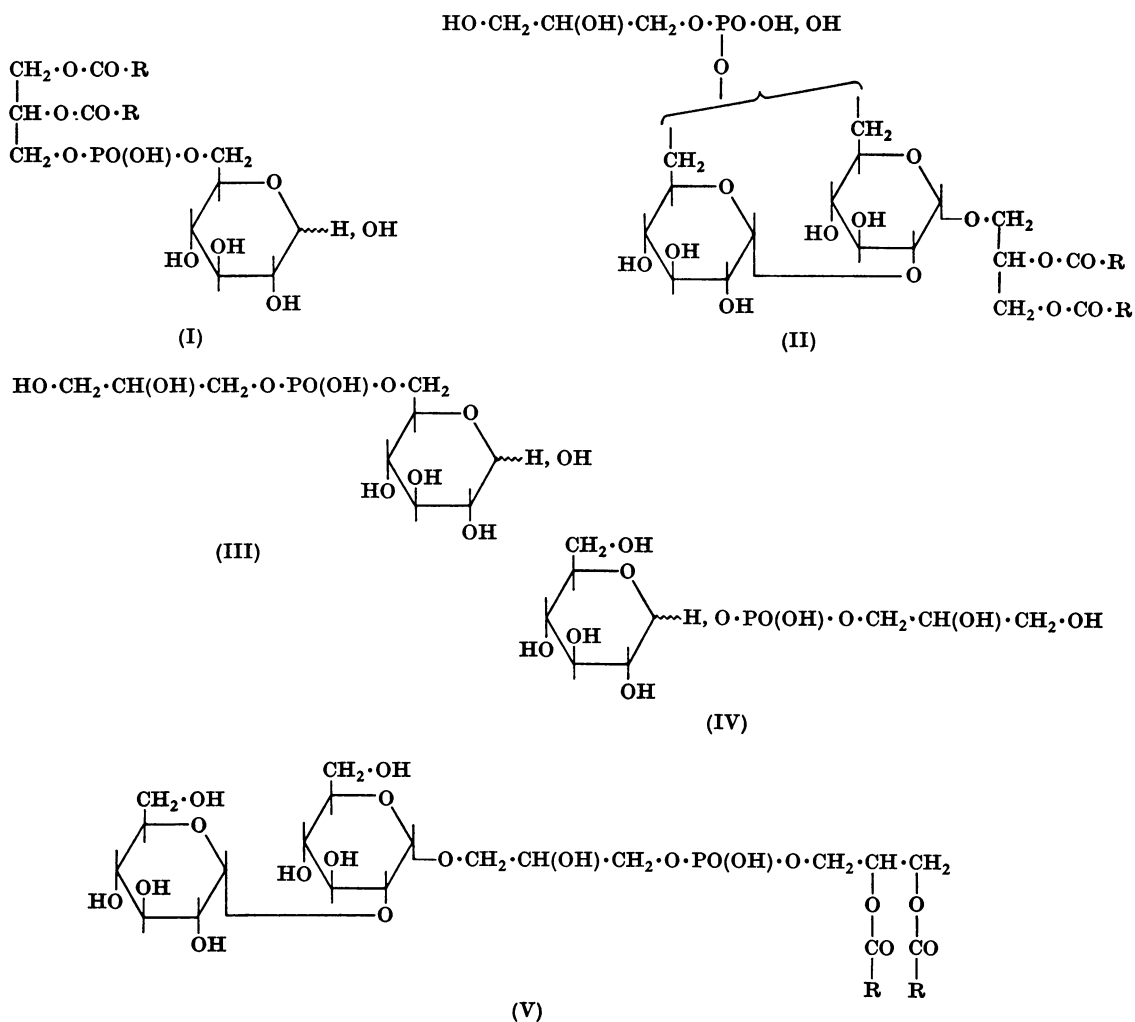
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Smith & Henrikson (1965) have reported the isolation from *Mycoplasma laidlawii* strain B of an unusual glucose-containing phospholipid for which a phosphatidylglucose structure (I) was tentatively proposed. Further studies have now established that this structure is untenable and an alternative structure (II), glycerylphosphoryldiglucoyl diglyceride, is now proposed.

The phospholipid was isolated from *M. laidlawii* and purified by the methods previously described (Shaw, Smith & Koostra, 1968). Hydrolysis with acid (*m*-hydrochloric acid at 100°C for 2h) gave a mixture of fatty acids, glucose and  $\alpha\beta$ -glycerophosphate. Deacylation of the phospholipid with sodium methoxide yielded a single water-soluble phosphate ester that did not co-chromatograph with a sample of glycerylphosphorylglucose (III), the deacylation product of phosphatidylglucose (I), synthesized from glycidol and glucose (cf. Brown, Hall & Higson, 1958), or with the isomeric compounds (IV) synthesized from glycidol and  $\alpha$ - or  $\beta$ -glucose 1-phosphate. Treatment of the deacylated phospholipid with sodium borohydride followed by acid hydrolysis still gave glucose and not glucitol, showing that the glucose was bound in the lipid through its reducing group. Hydrolysis with mild acid (*m*-hydrochloric acid at 100°C for 10 min) only partially released the glucose, precluding the presence of a sugar 1-phosphate linkage. The location of the glucose was established from studies of alkaline hydrolysis. Treatment with *m*-sodium hydroxide at 100°C for 1h completely hydrolysed the phospholipid to a mixture of fatty acids, diglucoylglycerol and  $\alpha\beta$ -glycerophosphate. No glucose-containing phosphate esters could be detected. The structure of the diglucoylglycerol was established as *O*- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)-*O*- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 1)-D-glycerol by the methods previously described for the identical glycoside obtained from the glycolipids of *M. laidlawii*

(Shaw *et al.* 1968). On oxidation the phospholipid consumed 4 mol of periodate and released 1 mol of formaldehyde/mol of phosphorus; the deacylated lipid consumed 5 mol of periodate and released 2 mol of formaldehyde/mol of phosphorus. All these results are consistent with the proposed glycerylphosphoryldiglucoyl diglyceride structure (II). No evidence is yet available to distinguish between the two possible isomeric structures containing a glycerophosphate residue located on the 6-hydroxyl group of either glucose residue. The phospholipid is completely resistant to hydrolysis by active preparations of phospholipases A, C and D (Verheij, Smith, Bonsen & van Deenen, 1970), indicating that the glycerophosphate residue is not esterified with fatty acids. Thus the generic term 'phosphatidyl' cannot be applied to this lipid.

Fischer & Seyferth (1968) have reported the isolation from *Streptococcus faecalis* and *Streptococcus lactis* of a glucose-containing phospholipid that yields a diglucoylglycerol and  $\alpha\beta$ -glycerophosphate on alkaline hydrolysis. Similar degradation products were obtained by Ishizuka & Yamakawa (1968) on alkaline hydrolysis of a phospholipid from *Streptococcus haemolyticus* strain D-58, and these authors suggested a diglucoylphosphatidylglycerol structure (V) analogous to the glucosaminylphosphatidylglycerol present in *Bacillus megaterium* (Op den Kamp, Bonsen & van Deenen, 1969) and *Pseudomonas ovalis* (Phizackerley, MacDougall & Francis, 1966). The results we have obtained are not consistent with a similar structure for the lipid from *M. laidlawii*. Oxidation with periodate of a diglucoylphosphatidylglycerol would not liberate formaldehyde and hydrolysis with alkali should yield detectable amounts of a diglucoylglycerol phosphate, which was not observed. Further studies may show that the glucose-containing phospholipids from streptococci are glycerylphosphoryldiglucoyl diglycerides, thereby emphasizing



the similarity in lipid composition previously shown between mycoplasmas and streptococci (Shaw *et al.* 1968; Plackett & Shaw, 1967).

While studying the biosynthesis of the glycolipids of *Streptococcus faecalis* A.T.C.C. 9790 Pieringer (1968) found that three glucose-containing lipids were formed sequentially from UDP-glucose and diglyceride. The first two were identified as monoglucosyl diglyceride and diglucosyl diglyceride, but the third lipid, which was apparently formed from diglucosyl diglyceride, was not identified. However, Pieringer (1968) did report that it was not a triglucosyl diglyceride, but that on deacylation it yielded an anionic water-soluble hydrolysis product. Although he did not identify the nature of the anionic group it seems probable that he was observing the synthesis of a glycerylphosphoryldi-

glucosyl diglyceride. The most likely biosynthetic route would be the addition of a glycerophosphate residue to a diglucosyl diglyceride. This route may also be operative in *M. laidlawii*, as the required diglucosyl diglyceride is a major lipid component of this organism (Shaw *et al.* 1968).

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