A NEW PHOSPHOGLYCOLIPID FROM STREPTOCOCCUS LACTIS [+)]

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Summary: One of the main phospholipids, isolated from Streptococcus lactis, was shown to contain glucose, glycerol, fatty acid ester and phosphorus in a molar ratio of about 2:2:3:1. Its structure has been established as the tri-O-acyl derivative of 1-O-[6'-(sn-glycero-3"-phosphoryl)-2'-O-(α-*D*-glucopyranosyl)-α-*D*-glucopyranosyl]-glycerol, in which each of the glycerol moieties is substituted with at least one fatty acyl residue.

Streptococcus faecalis and Streptococcus lactis have been suggested to contain chromatographically identical phosphoglycolipids, which seemed to be structurally related to the widespread dihexosyldiglycerides (2). In the present work the structure of the compound from S.lactis has been studied.

Methods: The analytical methods were essentially the same as previously described (2): carbohydrate by an anthrone procedure, glucose by a hexokinase-glucose-6-P dehydrogenase assay, glycerol by means of glycerokinase and glycero-3-P dehydrogenase, ester by a hydroxamate method (3), phosphorus by the method of Schnitger et al.(4), periodate consumption by a spectrophotometric assay (223 nm), formaldehyde by a chromotropic acid method.

Acid hydrolysis for measuring glucose and P-ester-bound glycerol was carried out in 1N HCl at 100°C for 3 and 240 hrs.,respectively.

The following chromatographic methods were used to establish identity and/or homogenity: (a) Lipids by thinlayer (silica gel G): $CHCl_3$, CH_3OH, H_2O 65:35:6; $CHCl_3$, CH_3OH, CH_3COOH, H_2O 170:

[+)] A preliminary report of this work has been given (1).

:25:25:6; $CHCl_3$, CH_3OH, 7N NH_4OH 65:30:4. (b) Phosphate-containing degradation products and dihexosylglycerol by thin layer (cellulose) and paper chromatography: phenol, H_2O, CH_3COOH, ethanol 75:33:4.5:4.5; propanol, 10N NH_4OH 3:2; isopropanol, 7N NH_4OH 7:4. (c) Hexoses and polyols by thin layer (cellulose): acetic ester, pyridine, H_2O 4:2:4 (upper phase). (d) Polyols by thin layer (silica gel G): butanol, H_2O 9:1; isopropanol, acetic ester, H_2O 23:65:12. The methods for detecting the various compounds on chromatograms were given elswhere (2) with the exception of the reagent for phosphatids (5).

Results: The chloroform-methanol extract from Streptococcus lactis was purified on sephadex from nonlipid contaminants and fractionated on DEAE-cellulose (acetate) as described previously (2). The fraction of the anionic phospholipids, about 40% of the total lipid, was separated on $NaHCO_3$-treated silic acid (6) by a discontinous gradient of chloroform(C)-methanol(M) into cardiolipin and phosphatidylglycerol (C:M,6:1) and a ninhydrin-negative compound with the staining properties of a phosphoglycolipid (C:M,3:1). The latter was further purified by rechromatography in the same system, and thereafter proved to be homogenous on thin layer chromatography. It accounted for about 27% of the phospholipids (w/w).

The molecular composition of the phosphoglycolipid is given in table 1. For structural analysis the lipid was deacylated un-

TABLE I: ANALYSIS OF THE PURIFIED PHOSPHOGLYCOLIPID

	Carbohydrate	Glucose	Glycerol	Ester	Phosphorus
µmole/mg	1.32	1.28	1.30	1.93	0.65
molar ratio	2.06	2.00	2.03	3.01	1.01

der mild alkaline conditions (7). The water-soluble product contained glucose, glycerol and phosphorus in a molar ratio of 1.98: 1.97: 1.00. It was unaffected by treatment with phospho - monoesterase, showing that the phosphate is in a diester linkage.

By strong alkaline hydrolysis (1N NaOH, 100°C, 3 hrs.) the compound was completely split into glycerophosphates and a phosphate-negative carbohydrate-containing compound. The products were separated by paper chromatography (isopropanol, 7N NH_4OH 7:4).

The phosphate-free compound showed glucose and glycerol in molar proportions of 1.92:1.00. As after strong alkaline treatment the carbohydrate was completely recovered, all the glucose must be in glycosidic bonds. The diglucosylglycerol had a molecular rotation of +65.600°, and was completely hydrolyzed by α-glucosidase (yeast, Boehringer) into glucose and glycerol, thus establishing the α-configuration for both the glucosidic bonds. The glycoside consumed 4.16 moles of periodate producing 1.02 mole formaldehyde; after acid hydrolysis neither glucose nor glycerol was detected. Finally the periodate oxidized glycoside was reduced with $NaBH_4$, and hydrolyzed with HCl: 2.00 moles glycerol were found per mole glycoside; on thinlayer chromatography, besides glycerol, ethylene glycol but no erythritol was detected. The glycoside is thus characterized as 1-O[2'-O-(α-*D*-glucopyranosyl)-α-*D*-glucopyranosyl]-glycerol, i.e. 1-O-α-koji - biosyl-glycerol.

The two phosphorus-containing compounds, isolated after strong alkaline hydrolysis, were identified by chromatography and analysis as α- and β-glycerophosphate (glycerol, P, vicinal hydroxyls 1.00:1.00:0.91 and 1.00:1.04:0,02, respectively). The α-glycerophosphate, about 40% of the total P, was shown to be

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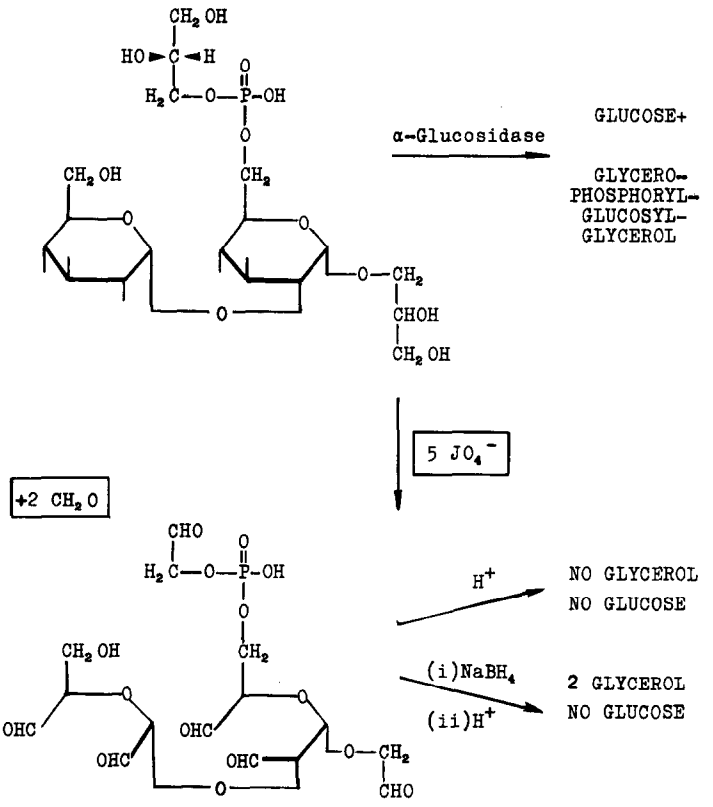

oxidation with $NaJO_4$: 1.90 moles/mole P).

Summarizing the results the deacylation product has the structure 1-O-[6'-(sn-glycero-3"-phosphoryl)-2'-O-(α-*D*-glucopyranosyl)-α-*D*-glucopyranosyl]-glycerol, and the lipid is the tri-O-acyl derivative of it. On oxidation of the intact lipid with periodate (in $CHCl_3$, ethanol, H_2O 2:3.5:1.5) no formaldehyde (0.07 mole/mole P) was found suggesting that each of the glycerol moieties is substituted with at least one fatty acyl residue.

The described structure is different from that of the few heretofore known bacterial phosphoglycolipids which are derivatives of phosphatidylglycerol (9, 10, 11). It may be of interest that α-kojibiosyl-diglyceride, a possible precursor of the new lipid, was also found in S.lactis (1).

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