Glucolipids from *Mycoplasma laidlawii* and *Streptococcus* MG

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During a study of the biosynthesis of lipids by *Mycoplasma* organisms (Plackett, 1967), it was found that the saprophytic *M. laidlawii* incorporated ^{14}C into non-acidic lipids when grown with [^{14}C]glucose. The present communication describes the isolation and characterization of the major non-acidic lipid, and of a very similar compound from *Streptococcus* MG.

Methods. M. laidlawii (strain B) was grown in BVF-OS medium (Turner, Campbell & Dick, 1935) containing 44μc of [G-^{14}C]glucose/l. The organisms were harvested by centrifugation and washed in cold 0·02M-phosphate buffer, pH7·0 ($Na^+ + K^+$), containing $MgSO_4$ (0·01M) (Plackett, 1967). *Streptococcus* MG (N.C.T.C. 8037) was grown in Todd–Hewitt broth and washed in cold 0·9% NaCl solution (Marmion, Plackett & Lemcke, 1967). Lipids were extracted from *M. laidlawii* with 19vol. of chloroform–methanol (2:1, v/v) (Plackett, 1967) and from *Streptococcus* MG by the procedure of Vorbeck & Marinetti (1965). Lipid extracts were washed by partition with 0·1M-KCl (0·2vol.), dried over Na_2SO_4 and fractionated on columns of DEAE-cellulose (Rouser, Kritchevsky, Heller & Lieber, 1963). Non-acidic lipids were eluted with neutral chloroform–methanol mixtures (100:1 and 7:3, v/v), and were rechromatographed on columns of silicic acid (Mallinckrodt, 100 mesh).

Thin-layer chromatography was carried out at room temperature on silica gel G plates with the solvent system chloroform–methanol–water (65:25:4, by vol.). Spots were located by spraying with 10N-H_2SO_4 or with the phenol–H_2SO_4 reagent of Gray (1965). The ^{14}C-labelled compounds were located by radioautography. Lipids were also chromatographed on Whatman SG81 paper in di-isobutyl ketone–acetic acid–water (40:20:3, by vol.) at 3°. Rhodamine 6G and the periodate–Schiff reagents (Vorbeck & Marinetti, 1965) were used to reveal the spots. Methanolysis of lipids was carried out under N_2 at room temperature with a mixture of chloroform and methanolic 0·2N-NaOH (2:1, v/v) at room temperature. After 1hr. at room temperature 0·2vol. of aq. 0·4N-acetic acid was added. The aqueous phase was separated, and the chloroform phase washed with water, dried over Na_2SO_4 and retained for analysis of the fatty acid methyl esters. The water-soluble deacylation product was recovered from the aqueous layers after deionization. Water-soluble substances were chromatographed on Whatman no. 1 paper. Spots were revealed with $AgNO_3$ (Dedonder, 1952). Fatty acid esters were determined by the method of Antonis (1960) and by gas–liquid chromatography on columns packed with 15% ethylene glycol succinate on Diatoport S. Methylpentadecanoate was the standard. Hexoses were determined by the phenol–H_2SO_4 method (Hodge & Hofreiter, 1962) and with the Glucostat reagents (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). Glycerol was determined by the method of Wieland (1963) and 1,2-glycols by the periodate–chromotropic acid method (Plackett, 1964). Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. The kaolin agglutination test of Takahashi (1962) was used to study the serological reactivity of the glycolipids. Methanol solutions of the lipid were mixed with 20vol. of warm 0·9% NaCl containing tris–maleate buffer and EDTA (Takahashi, 1962). Samples of the resulting dispersions (1vol.) were mixed with 2vol. of kaolin suspension (1mg./ml.). Then 0·1ml. volumes of serum dilutions in the same buffered diluent and 0·1ml. portions of the treated kaolin suspensions were mixed in plastic wells and kept at room temperature overnight. Complement-fixation tests were carried out as described by Marmion *et al.* (1967). Samples of the lipids (80–150μg.) were mixed with egg lecithin (50μg.) in 0·1ml. of ethanol before dispersion in 1·0ml. of veronal-buffered 0·9% NaCl.

Results and discussion. The neutral lipids of *M. laidlawii*, which accounted for 39% of the ^{14}C incorporated into the lipid fraction, were rechromatographed on silicic acid columns with chloroform–acetone mixtures (3:1 and 1:1, v/v) and acetone for elution. Two minor components (A and B), accounting for 8% of the ^{14}C in the lipids, were eluted together. These gave a single spot (R_F 0·91) on thin-layer chromatograms, but two periodate–Schiff-positive spots [R_F 0·52 (component A) and 0·58 (component B)] on Whatman SG81 paper. Their mobilities indicate that they may be monosaccharide derivatives, but they were not identified.

They were followed by the major neutral component (C), which gave a single spot in all three chromatographic systems. It was also periodate–Schiff-positive and accounted for 31% of the ^{14}C in the lipid fraction. The R_F value of component C was 0·80 on thin-layer plates and 0·35 on Whatman SG 81 paper.

Methanolysis of component C gave fatty acid methyl esters and a non-reducing water-soluble product containing a 1,2-glycol group (1·05 equiv. of 1,2-glycol/2·00 moles of hexose). The yield of fatty esters (per 2·00 moles of hexose) was 2·08 moles by the hydroxamate method and 1·83 moles by gas–liquid chromatography. R_{Glc} values for the water-soluble product were 0·44 in butan-1-ol–pyridine–water (6:4:3, by vol.), 0·69 in butan-1-ol–acetic acid–water (5:2:3, by vol.) and 0·93 in propan-2-ol–aq. NH_3 (sp.gr. 0·88)–water (35:3:15, by vol.). It was hydrolysed in N-H_2SO_4 at 100° for 4 hr. to give glucose and glycerol, identified by paper chromatography and by reaction with glucose oxidase and with glycerokinase plus glycerophosphate dehydrogenase. The recovery of glycerol was 0·95 mole/2·00 moles of hexose. These data are consistent with a diglucosyl diglyceride structure for component C, in agreement with the findings of Shaw & Smith (1967), who reported the identification of deacylation products of *M. laidlawii* glycolipids as *O*-α-D-glucopyranosyl-(1→1)-glycerol and *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1)-D-glycerol.

The neutral lipid fraction from *Streptococcus* MG contained components chromatographically indistinguishable from components A, B and C. That migrating like component C was the most abundant, and was obtained in a yield representing 0·14% of the wet weight of the cells. Fatty acid methyl esters were determined by gas–liquid chromatography after methanolysis. The yield was 1·62 moles/2·0 moles of hexose. There were minor differences in fatty acid composition. The water-soluble deacylation product (0·98 mole of 1,2-glycol/2·00 moles of hexose) was chromatographically indistinguishable from that obtained from *M. laidlawii* in three solvent systems, and gave glucose and glycerol on acid hydrolysis.

That the diglucosyl lipids from the two organisms have similar configurations is also indicated by their serological cross-reactivity. A rabbit antiserum to *M. laidlawii* gave maximum serum titres (1:160 to 1:320) in the kaolin agglutination test with concentrations of either lipid down to about 1 μg./mg. of kaolin. Preinoculation serum from the same rabbit did not agglutinate the particles. Particles treated with digalactosylglyceride from spinach leaves were not agglutinated by either serum. In complement-fixation tests with antisera to *M. laidlawii* and to *Streptococcus* MG, the two glucolipids gave similar end points with either serum.

Although many of the glycosphingolipids found in animal tissues have been shown to function as haptens (Brady, 1966), little is known of the serological activity of the glycosylglycerides. The widespread occurrence of these compounds in bacteria, including *Mycoplasma* organisms, makes it probable that they are of some immunological significance.

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