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Biosynthesis of Glucosyl Diglycerides by *Mycoplasma laidlawii* Strain B

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Monoglucosyl diglyceride is synthesized from 1,2-diglyceride and uridine-5'-diphosphoglucose (UDP); diglucosyl diglyceride from monoglucosyl diglyceride, and uridine-5'-diphosphoglucose by membranes of *Mycoplasma laidlawii* strain B. All of these enzymatic activities reside in the membrane. Membranes solubilized by detergent action or succinylation and acetone powders of membranes were inactive. Requirements for Mg^{2+}, UDP, and appropriate lipid acceptor were demonstrated for biosynthesis of both glycolipids. Glucose-1-phosphate plus uridine triphosphate could replace the UDP requirement. A medium of relatively high ionic strength and a critical concentration of sodium lauryl sulfate stimulated biosynthesis of the monoglucosyl diglyceride. The optimal *p*H for both reactions was 8.0. A specificity for 1,2-diglyceride from the homologous organism was found for optimal synthesis of the monoglucosyl diglyceride, and a specificity for monoglucosyl diglyceride was found in the case of diglucosyl diglyceride synthesis. Both reactions were specific for UDP.

Glycosyl diglycerides are found among gram-positive bacteria (5, 8, 18, 21), some mycoplasmas (13, 14, 19) and in higher plants (2–4, 17). The specific structures of the glycolipids appear correlative to the taxonomic position of the bacteria (18). The absence of turnover of these lipids (P. F. Smith, *unpublished data*) as well as their abundance and membranous location suggest a structural role. The glycolipids of *Mycoplasma laidlawii* strain B have been identified as 1-[0-α-D-glucopyranosyl-(1 → 2)-0-α-D-glucopyranosyl]-2,3-diglyceride (DGDG) and 1-[0-α-D-glucopyranosyl]-2,3-diglyceride (19). The monoglycosyl diglyceride (MGDG) predominates in mycoplasmas (19), whereas the reverse is true among the bacteria (5, 18).

Biosynthesis of glycosyl diglycerides in the organisms examined thus far proceeds by the transfer of the hexose from the appropriate nucleotide sugar to the lipid acceptor, diglyceride, for the synthesis of the monoglycosyl lipid and monoglycosyl diglyceride in the case of the diglycosyl lipid (8, 10, 12). The biosynthesis of the glycolipids of *M. laidlawii* was examined to define further the lipid physiology of *Mycoplasma*.

MATERIALS AND METHODS

M. laidlawii strain B was grown in a liquid medium composed of 2% Tryptose (Difco), pre-extracted in the dry state with chloroform-methanol, 0.5% sodium acetate, and 0.5% glucose, added as a sterile solution to the autoclaved medium. The *p*H of the medium was 8.0 prior to sterilization. Each liter of medium was inoculated with 100 ml of a 16-hr-old culture. Cultures were incubated without shaking at 37 C for 16 hr at which time the organisms were concentrated in a Sharples centrifuge operating at 25,000 rev/min and sedimented at 0 C at 20,000 × *g* in a Servall RC-2B centrifuge. A 16-hr incubation period was selected, since the culture was in the mid-exponential phase of growth. Higher yields are obtained by lengthening the incubation period but enzymatic activity was reduced or absent.

The unwashed pellets were suspended by trituration in 0.002 M tris(hydroxymethyl)aminomethane-(Tris)-maleate buffer, *p*H 8.0, at room temperature. Lysis was immediate and complete. The lysate was sedimented at 39,000 × *g* for 25 min at 0 C. The sedimented membrane appeared as clear yellow pellets in contrast to the opaque pellets of intact organisms. Approximately 75% of the total protein but none of the lipid appeared in the supernatant fluid. The membranes were washed twice in 0.002 M buffer and resuspended in a volume sufficient to give a protein concentration of about 25 mg per ml. Fresh membranes were prepared daily.

Acetone powders of membranes were prepared by dropping 5 ml of suspension into 100 ml of acetone at −20 C, followed by sedimentation at 8,000 × *g*. The sediment was dried under a stream of nitrogen. In some instances an additional washing with acetone at −20 C was performed. The supernatant acetone solution was dried in vacuo at 30 C. Solubilization of membranes was achieved either by detergent lysis or

succinylation. Sodium dodecyl sulfate (SDS), 4 μmoles per mg of protein, caused complete solubilization as determined by nonsedimentability at 39,000 × *g* for 25 min; for Nonidet, P-40 (Shell), a polyethylated tertiary-octyl phenol, 1% final concentration was sufficient. Both nondialyzed and extensively dialyzed preparations were used. Succinylation was accomplished by the procedure described by Haas (6). Solid succinic anhydride was added with stirring over a period of 1 hr to the membrane suspended in *p*H 8.0, 0.2 M Tris-maleate buffer until a 20-fold excess over the molar concentration of free amino groups was obtained. The *p*H was held between 7.5 and 8.0 by incremental additions of 1 N potassium hydroxide. Stirring was continued for 15 min after the *p*H remained constant. The clear yellow solution was dialyzed for 24 hr at 4 C against multiple changes of 0.2 M Tris-maleate buffer, *p*H 8.0.

1,2-Diglycerides were prepared from egg phosphatidyl choline and *M. laidlawii* phospholipids. Egg phosphatidylcholine was prepared from fresh egg yolks by the procedure outlined by Ansell and Hawthorne (1). Purity was assessed by thin-layer chromatography. Mixed phospholipids from *M. laidlawii* were prepared as follows: 5 g of lyophilized organisms were extracted three times with 200 ml of chloroform-methanol (2:1, v/v). After drying the pooled extracts in vacuo at 40 C, the total lipids were purified by passage through Sephadex G-25 (22). The eluate again was dried in vacuo and the major lipid classes separated on a silicic acid column (20). Neutral lipids were eluted with chloroform, glycolipids with acetone, and phospholipids with chloroform-methanol (1:10, v/v). The column eluates were dried in vacuo. The egg phosphatidylcholine or the mixed phospholipids of *M. laidlawii* were emulsified in 10 ml of 0.1 M Tris-maleate buffer, *p*H 7.2, by mild sonic treatment (30 to 60 sec in a 10-kc Raytheon oscillator). To the emulsions was added 2 to 5 mg of phospholipase C, prepared from *Bacillus cereus* ATCC 10987 by the procedure of Ottolenghi (11), 1 to 2 mg of zinc chloride and 0.2 ml of diethyl ether. After 60 hr at room temperature, the ether was removed in vacuo; the mixture was lyophilized, and the dry residue was extracted with chloroform-methanol (2:1, v/v). Diglycerides were separated from unhydrolyzed phospholipid by passage through a column (1 × 4 cm) of silicic acid (Unisil; Clarkson Chemical Co., Williamsport, Pa.). Diglycerides were eluted with 200 ml of chloroform and dried in vacuo. This material yielded one iodine and water-positive spot on Silica Gel H (E. Merck AG, Darmstadt, Germany) thin-layer chromatograms developed in hexane-ether-glacial acetic acid (70:30:1, v/v). The R_F was 0.27 compared to 0.35 for 1,3-diolein. The concentration of diglyceride was assayed by the hydroxamate method of Rapport and Alonzo (15). ^{3}H-Diglyceride was prepared in the same way except the starting material consisted of cardiolipin (Sylvana Chemical Co., Orange, N.J.) tritiated by reduction of the unsaturated fatty acid residues. Tritiation was performed by New England Nuclear Corp., Boston, Mass. Excess tritium was removed by extensive washing. Specific activity was 1.5 mc per mg.

MGDG was prepared from *M. laidlawii*. The acetone eluate from silicic acid column chromatography described previously was subjected to thin-layer chromatography on 1-mm thick Silica Gel H. Development was performed in chloroform-methanol (9:1, v/v). The area containing MGDG was scraped from the plates and eluted with chloroform-methanol (2:1, v/v). Two successive chromatograms yielded a pure product with a ratio of glucose to fatty acid ester of approximately 1:2 (19). Several lots were prepared. MGDG-glucose-^{14}C was prepared by carrying out biosynthesis of this compound from diglyceride and uridine-5′-diphosphate (UDP)-glucose-^{14}C as described below but in larger quantity. Purification was accomplished as described for the unlabeled MGDG.

1,2-Diolein, 1,2-dipalmitin, 1,2-dimyristin, 1,2-distearin, and monogalactosyl diglyceride from beef heart were obtained from either Hormel Institute, Austin, Minn. or Applied Sciences Laboratory, State College, Pa. Protein was determined by the method of Lowry et al. (9), free amino groups by the method of Rosen (16), and glucose by the anthrone reaction or glucose oxidase or both (Worthington Biochemical Corp., Freehold, N.J.). Unlabeled and ^{14}C-labeled hexose nucleotides were obtained from International Chemical and Nuclear Corp., City of Industry, Calif.

MGDG and DGDG were synthesized enzymatically as follows. An emulsion of MGDG or diglyceride was prepared in water or 0.2 M Tris-maleate buffer, *p*H 8.0, to give a concentration of 10 μmoles per ml. To 0.1 ml of this emulsion were added UDP glucose-^{14}C, 1.0 μmole (0.5 μc); magnesium chloride, 20 μmoles; SDS, 0.5 μmole; Tris-maleate buffer (*p*H 8.0), 150 μmoles, and membrane suspension, ca. 3 to 4 mg protein in a total volume of 1.0 ml. After mixing, incubation was at 37 C for 60 min. The reaction was not linear in all cases over the 60-min time period, so the values expressed represent total synthesis. The reaction was stopped by quick freezing at −20 C. After lyophilization, the lipids were extracted three times with 5 ml, 2 ml, and 2 ml, respectively, of chloroform-methanol (2:1, v/v), and the pooled extracts were dried in vacuo. The total lipids were redissolved in 0.2 ml of chloroform-methanol (2:1, v/v) and applied to 1-mm thick plates of Silica Gel H. Tubes were rinsed twice with an additional 0.1 ml of solvent. The plates were developed in chloroform-methanol, 9:1 (v/v) for MGDG separation, and 8:2 (v/v) for DGDG separation. The appropriate areas on the plates were detected by exposure to iodine vapor and scraped into vials for counting. Radioactivity was measured as previously described (7). The amounts of lipids formed were determined by calculations based upon the specific activity of the UDP-glucose-^{14}C added. This method was valid since direct incorporation of glucose from the nucleotide sugar occurred. Identifications of the products as MGDG and DGDG were made by (i) measuring the specific radioactivity of glucose in the newly synthesized lipid and relating it to the specific activity of the UDP-glucose-^{14}C added, (ii) migration on thin-layer chro-

matograms, and (iii) measuring ratio of glucose to fatty acid ester.

RESULTS

Studies on the biosynthesis of the glucosyl diglycerides in *M. laidlawii* were complicated by the inability to remove endogenous lipids and still retain enzymatic activity. Hence endogenous lipid acceptors allowed a higher base line activity than was desired. Nevertheless, specific requirements for the reactions could be assessed. Optimal activity required the addition of magnesium ions, UDP-glucose, and *M. laidlawii* membranes for both reactions, 1,2-diglyceride for MGDG synthesis, and MGDG for DGDG synthesis (Table 1). The reaction was enzyme-catalyzed as judged from the heat lability of the membrane preparation, the absence of synthesis in the absence of membranes (Table 1), and the proportionate increase in product formed with increase in protein concentration (Fig. 1). A critical concentration of membrane appeared necessary before monoglucosyl diglyceride formation occurred, probably reflecting the degree of sensitivity of the techniques employed (Fig. 1A). The enzymatic activities of membrane preparations varied somewhat in different lots. This variation is reflected in the tabular data. One cause of this variation was found to be associated with the use of lipid-free PPLO Serum Fraction (Difco) as an aid in emulsification of MGDG and diglyceride. As seen in Table 2, increasing concentrations resulted in decreased glycolipid synthesis. After discovery of this effect, the serum fraction was deleted from the reaction mixture. The data for DGDG synthesis found in Tables 4–7 and Fig. 4 and 5B were obtained in the presence of Serum Fraction.

The site of biosynthesis of both glycolipids is associated solely with the membrane fraction (Table 3). The location of the enzymatic activities in membranes thwarted efforts at purification. Acetone powders were completely devoid of activity even upon readdition of the acetone-soluble lipids. Solubilization of the membranes with anionic or nonionic detergents followed by extensive dialysis likewise resulted in inactive preparations. Succinylation as a means of solubilization gave inactive preparations even if sulfhydryl groups were protected during succinylation with p-chloro-mercuribenzoic acid.

The saturation curves for the reactants are shown in Fig. 2 and 3. The affinity for UDP-glucose appeared to be slightly higher in the synthesis of DGDG than in synthesis of MGDG. Similar affinities for diglyceride and MGDG were observed but concentrations of diglyceride above 1 μM greatly inhibited formation of MGDG.

TABLE 1. *Requirements for biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*

Condition	Monoglucosyl diglyceride[a]	Diglucosyl diglyceride[a]
Complete	18.5	3.8
Minus		
Diglyceride	0.3	
Monoglucosyl diglyceride		0.6
Uridine-5'-diphosphate glucose	0.01	0.01
$MgCl_2$	0.6	1.4
Sodium dodecyl sulfate	9.8	3.8
M. laidlawii membrane	0.01	0.01
Complete, heat-inactivated membrane	0.01	0.01
Complete, EDTA (10 μmoles)	2.3	0.9

[a] Expressed in nanomoles per milligram of protein in 60 min.

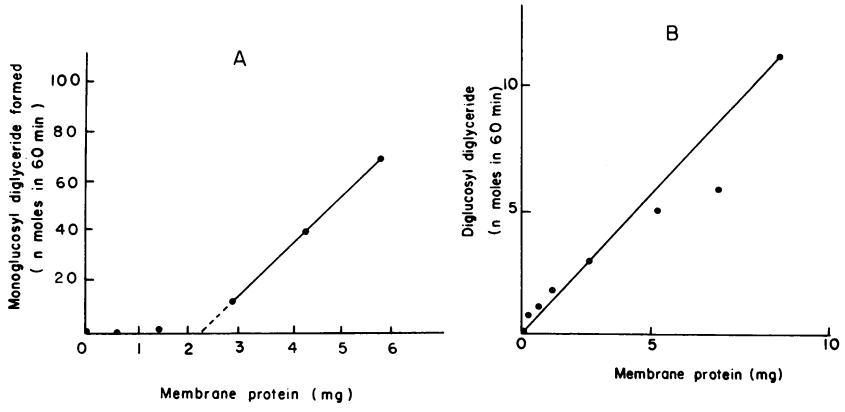

FIG. 1. *Effect of membrane protein concentration on biosynthesis of glucosyl diglycerides by M. laidlawii.*

Under optimal conditions, about 12% incorporation of UDP-glucose into MGDG was noted, but only 2% incorporation into DGDG occurred. By using sonically treated organisms, UDP-glucose could be substituted with glucose-1-phosphate and uridine triphosphate (Fig. 4) or with glucose, adenosine triphosphate, and uridine triphosphate, although activity was reduced. Absence of the nucleotide triphosphates almost negated glycolipid biosynthesis. These results indirectly suggest a capability for UDP-glucose biosynthesis by the organisms.

The optimal *p*H for biosynthesis of both glycolipids was 8.0 (Table 4). Stimulation of DGDG synthesis occurred only with Mg^{2+}. A variety of cations exhibited stimulatory activity for the biosynthesis of MGDG (Table 5). Lower or higher concentrations of cations other than

TABLE 2. *Effect of lipid-free PPLO Serum Fraction on biosynthesis of diglucosyl diglyceride*

Serum fraction (mg)	Diglucosyl diglyceride formed[a]
0	8.8
1	5.0
5	3.7
10	2.9

[a] Expressed as nanomoles per milligram of protein in 60 min.

TABLE 3. *Site of biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*[a]

Cell fraction	Per cent of total cellular protein	Monoglucosyl diglyceride formed	Diglucosyl diglyceride formed
Lysate	100	4.47	0.49
Membrane	30.5	14.83	2.33
Supernatant fluid	69.5	0.05	0

[a] Expressed as nanomoles per milligram of protein in 60 min.

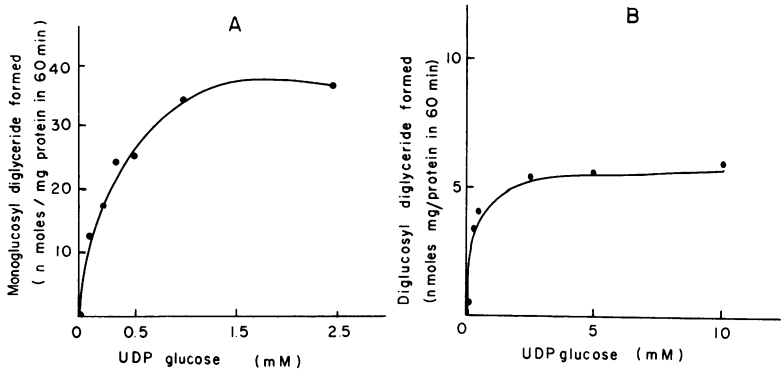

FIG. 2. *Effect of concentration of uridine-5'-diphosphoglucose on biosynthesis of glucosyl diglycerides by M. laidlawii.*

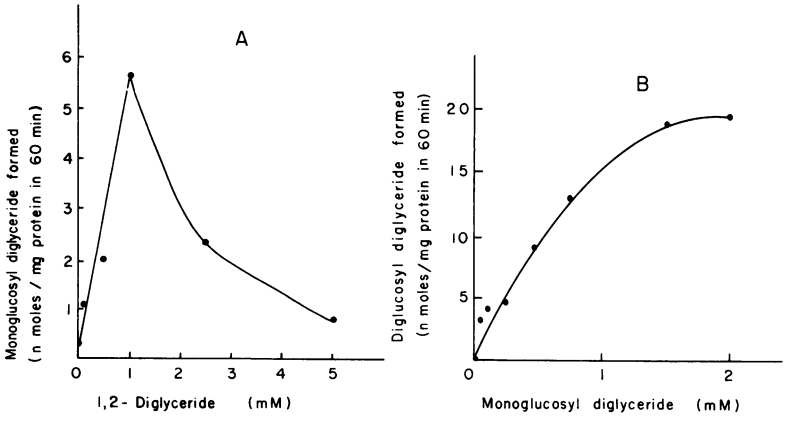

FIG. 3. *Effect of concentration of diglyceride and of monoglucosyl diglyceride on biosynthesis of monoglucosyl diglyceride and diglucosyl diglyceride, respectively, by M. laidlawii.*

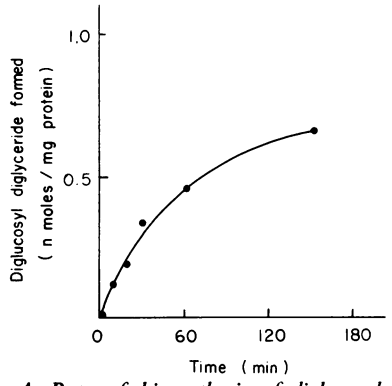

FIG. 4. *Rate of biosynthesis of diglucosyl diglyceride from glucose-1-phosphate, uridine triphosphate, and monoglucosyl diglyceride by M. laidlawii. Conditions of reaction are given , except for substitution of 1 μmole of UL-^{14}C-glucose-1-phosphate and 2 μmoles of uridine triphosphate for uridine-5'-diphosphoglucose.*

TABLE 4. *Effect of pH on biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*[a]

pH	Monoglucosyl diglyceride formed	Diglucosyl diglyceride formed
5.0	0.14	0.06
6.0	0.21	0.4
6.5	9.70	
7.0	10.53	0.9
7.5	11.77	1.1
8.0	25.70	1.5
8.5	24.87	1.1
9.0	3.84	0.7
10.0	3.77	0.3

[a] Expressed as nanomoles per milligram of protein in 60 min.

TABLE 5. *Effect of various cations and of various concentrations of Mg^{2+} on biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*[a]

Cation	Concn (μmoles)	Monoglucosyl diglyceride formed	Diglucosyl diglyceride formed
None		3.13	1.3
Mn^{2+}	20	7.32	1.2
Ca^{2+}	20	9.54	1.2
Na^{+}	20	9.21	1.2
Mg^{2+}	1	19.33	
	3	16.89	
	7	19.70	
	10	16.60	
	20	18.51	3.8

[a] Expressed as nanomoles per milligram of protein in 60 min.

Mg^{2+} could have different effects. Further evidence of the necessity for a cation is the inhibitory action of ethylenediaminetetraacetic acid (Table 1). The anionic detergent SDS markedly stimulated biosynthesis of MGDG but had no apparent effect on DGDG formation (Table 6). This result is probably a reflection of the solubilizing effect of the detergent on the more water-insoluble diglyceride. No such effect would be necessary for the MGDG, which is more water soluble by virtue of containing the polar glucose radical. The stimulatory effect of the detergent on MGDG synthesis occurred over a narrow range, with higher concentrations completely abolishing activity. Similar results were obtained when the ionic strengths of the reaction mixtures were varied (Table 7). MGDG synthesis was more acutely affected, although both reactions were inhibited at high ionic strength. Again these results probably are a reflection of the physical state of the lipids in the reaction mixture.

The specificities of the reactions for various precursors are shown in Table 8. Only UDP glucose served as hexose donor for MGDG synthesis. No incorporation of mannose from guanosine-5'-diphosphate mannose or galactose from UDP galactose was observed. Synthetic 1,2-diglycerides not only were incapable of serving as glucose acceptors but also caused a 50% reduction in endogenous incorporation of glucose into glycolipids. 1,2-Diglyceride derived

TABLE 6. *Effect of various concentrations of sodium dodecyl sulfate on biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*[a]

Sodium dodecyl sulfate (μmoles)	Monoglucosyl diglyceride formed	Diglucosyl diglyceride formed
0	9.78	1.3
0.05	18.65	1.1
0.25	25.06	1.3
0.5	20.89	1.1
2.5	0	1.2
5.0	0.05	1.5

TABLE 7. *Effect of ionic strength on biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*

Concn of buffer	Monoglucosyl diglyceride formed	Diglucosyl diglyceride formed
M		
0.0002	4.66	2.1
0.002	4.68	1.5
0.02	17.52	1.7
0.10	16.41	1.4
0.20	14.38	0.9
1.00	3.74	0.4

from egg yolk phosphatidylcholine permitted less than 10% of the incorporation of glucose into MGDG as seen with homologous 1,2-diglyceride. No doubt the nature of the fatty acid residues accounts for these results. Commercial monogalactosyl diglyceride failed to serve as glucose acceptor.

The time courses of biosynthesis of the two glycolipids are shown in Fig. 5. In both cases, the rates are relatively linear over the first 20 to 30 min.

The data pertinent to identification of the products of biosynthesis are shown in Table 9. The incorporation of ^{14}C-MGDG into DGDG amounted to 1.7% of total available lipid acceptor compared to 1.8% incorporation of UDP-glucose-^{14}C. The specific activities of the lipids in terms of glucose and of MGDG in terms of ^{3}H-diglyceride are equivalent within experimental error to the specific activities of the starting compounds, indicating direct incorporation. All of the radioactivity of the ^{14}C-glucose-labeled lipids was found associated with their deacylation products.

TABLE 8. *Specificity of various precursors on biosynthesis of glucosyl diglycerides by Mycoplasma laidlawii B*

Precursor	Concn	Monoglycosyl diglyceride formed[a]	Diglycosyl diglyceride formed[a]
	μM		
UDP glucose	0.5	25.36	1.7
	2.5	36.25	3.0
	0.5	0	0
UDP galactose	2.5	0	0
Guanosine-5'-diphosphate mannose	0.5	0	0.01
	2.5	0	0.07
1,2-dimyristin	1.0	0	0
1,2-dipalmitin	1.0	0	0
1,2-distearin	1.0	0	0
1,2-diolein	1.0	0	0
1,3-diolein	1.0	0	
1,2-diglycerides from egg yolk phosphatidylcholine	1.0	3.03	
1,2-diglycerides from *M. laidlawii* phospholipids	1.0	36.25	
Monogalactosyl diglyceride	2.0		0

[a] Expressed as nanomoles per milligram of protein in 60 min.

DISCUSSION

The biosynthesis of mannosyl diglycerides by *Micrococcus lysodeikticus* (8), of glucosyl diglycerides by *Streptococcus faecalis* (12), and of galactosyl diglycerides by various plants (10) has been described. The properties of the system capable of biosynthesis of monoglucosyl di-

TABLE 9. *Identification of products of glycolipid biosynthesis by Mycoplasma laidlawii B*

Property	Monoglucosyl diglyceride	Diglucosyl diglyceride
R_F, Silica Gel H Chloroform-methanol (9:1, v/v)	0.6	0.2
R_F, deacylation product *n*-Butanol-pyridine-water (6:4:3, v/v)	0.40	0.18
Fatty acid ester-glucose	2.1:1.0	2.0:1.8
Specific activity glucose-^{14}C in glycolipid-specific activity glucose-^{14}C in UDP-glucose[a]	1.00 (1.00)[b]	0.46 (0.50)
Specific activity diglyceride-^{3}H in glycolipid-specific activity diglyceride-^{3}H added[a]	0.82 (1.00)	

[a] Endogenous controls were subtracted prior to calculation of specific activities.

[b] Calculated values are expressed parenthetically.

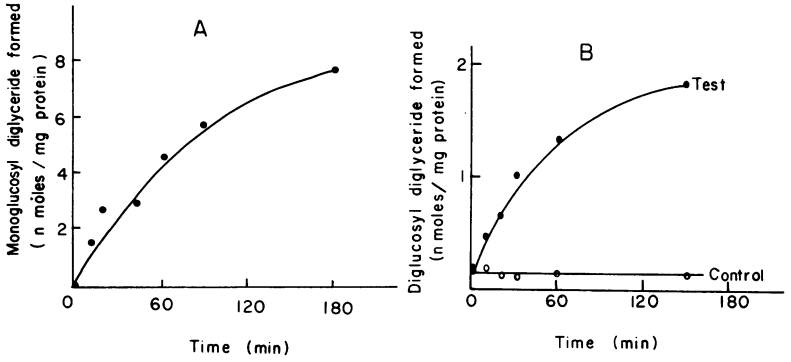

FIG. 5. *Rates of biosynthesis of glucosyl diglycerides by M. laidlawii.*

glyceride by *M. laidlawii* are similar to those for biosynthesis of monomannosyl diglyceride (8) and monoglucosyl diglyceride (12) by bacteria. Mg^{2+} is stimulatory, there exists a high degree of specificity for diglyceride, and biosynthesis functions best in a medium of relatively high ionic strength. The enzymes carrying out biosynthesis of all monoglycosyl diglycerides thus far examined are membrane-associated. Some differences do exist. For example, the system of *M. lysodeikticus* possesses an absolute requirement for fatty acids or detergents. Such a requirement by *M. laidlawii* in this study and by *S. faecalis* (12) probably was not apparent because lipid-containing membranes rather than an acetone powder were used as the enzyme source. The specificity of nucleotide sugar by *M. laidlawii* is similar to the specificity observed with *S. faecalis*.

Very little work has been reported on the biosynthesis of diglycosyl diglycerides. This system in *M. laidlawii* differs from those in *M. lysodeikticus* and plants by its association with the membrane in contrast to its association with the soluble form in the latter two cases. The particulate fraction of *S. faecalis* carries out biosynthesis of diglucosyl diglyceride (12).

The existence of distinct enzymes for the biosynthesis of mono- and diglycosyl diglycerides is less certain for *M. laidlawii* and presumably *S. faecalis* (12). In the case of *M. lysodeikticus* and plants, the enzymatic activity for synthesis of monoglycosyl diglyceride is associated with the particulate fraction, whereas the activity for synthesis of diglycosyl diglyceride is soluble. In *M. laidlawii*, both activities are membrane-associated. Several distinctions do exist between the two activities in *M. laidlawii*. Detergent stimulates only monoglucosyl diglyceride biosynthesis, and ionic strength of the medium has a greater effect on synthesis of this lipid. This is probably a phenomenon associated with the physical state and aqueous solubility of the diglyceride acceptor. The affinities of the two systems for nucleotide sugar differ only slightly. These results may signify some distinction between the enzymes. The relative biosynthetic activities for the two glycolipids coincide with the relative quantities found in *M. laidlawii*. The content of monoglucosyl diglyceride in the organism and its rate of biosynthesis are twice that of the diglucosyl diglyceride.

These studies represent an analysis of the terminal biosynthetic reactions. The source and biosynthetic mechanisms of the precursors of these lipids remain to be elucidated.

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LITERATURE CITED

1. Ansell, G. B., and J. N. Hawthorne. 1964. Phospholipids, p. 89. Elsevier Publishing Co., Amsterdam.
2. Benson, A. A., R. Wiser, R. A. Farrari, and J. A. Miller. 1958. Photosynthesis of galactolipids. J. Amer. Chem. Soc. 80:4740.
3. Carter, H. E., R. A. Hendry, and N. Z. Stanacev. 1961. Wheat flour lipids. III. Structure of the mono- and digalactosylglycerol lipids. J. Lipid Res. 2:223–227.
4. Carter, H. E., K. Ohno, S. Nojima, C. L. Tipton, and N. Z. Stanacev. 1961. Wheat flour lipids. II. Isolation and characterization of glycolipids of wheat flour and other plant sources. J. Lipid Res. 2:215–222.
5. Cohen, M., and C. Panos. 1966. Membrane lipid composition of *Streptococcus pyogenes* and derived L form. Biochemistry 5:2385–2392.
6. Hass, L. F. 1964. Aldolase dissociation into subunits by reaction with succinic anhydride. Biochemistry 3:535–541.
7. Henrikson, C. V., and P. F. Smith. 1966. Conversion of mevalonic acid to γ,γ-dimethylallyl pyrophosphate by *Mycoplasma*. J. Bacteriol. 92:701–706.
8. Lennarz, W. J., and B. Talamo. 1966. The chemical characterization and enzymatic synthesis of mannolipids in *Micrococcus lysodeikticus*. J. Biol. Chem. 241:2707–2719.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
10. Ongun, A., and J. B. Mudd. 1968. Biosynthesis of galactolipids in plants. J. Biol. Chem. 243:1558–1566.
11. Ottolenghi, A. C. 1965. Phospholipase C from *Bacillus cereus*, a zinc-requiring metalloenzyme. Biochim. Biophys. Acta 106:510–518.
12. Pieringer, R. A. 1968. The metabolism of glyceride glycolipids. I. Biosynthesis of monoglucosyl diglyceride and diglucosyl diglyceride by glucosyltransferase pathways in *Streptococcus faecalis*. J. Biol. Chem. 243:4894–4903.
13. Plackett, P. 1967. Glucolipids from *Mycoplasma laidlawii* and *Streptococcus* MG. Biochem. J. 104:61C–62C.
14. Plackett, P. 1967. The glycerolipids of *Mycoplasma mycoides*. Biochemistry 6:2746–2754.
15. Rapport, M. M., and N. Alonzo. 1955. Photometric determination of fatty acid ester groups in phospholipides. J. Biol. Chem. 217:193–198.
16. Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. 67:10–15.
17. Rosenberg, A., J. Gouaux, and P. Milch. 1966. Monogalactosyl and digalactosyl diglycerides from heterotrophic, hetero-autotrophic, and photobiotic *Euglena gracilis*. J. Lipid Res. 7:733–738.
18. Shaw, N., and J. Baddiley. 1968. Structure and distribution of glycosyl diglycerides in bacteria. Nature (London) 217:142–144.
19. Shaw, N., P. F. Smith, and W. L. Koostra. 1968. The lipid composition of *Mycoplasma laidlawii* strain B. Biochem. J. 107:329–333.
20. Smith, P. F., and W. R. Mayberry. 1968. Identification of the major glycolipid from *Mycoplasma* sp., strain J as 3,4,6-triacyl-β-D-glucopyranose. Biochemistry 7:2706–2710.
21. Vorbeck, M. L., and G. V. Marinetti. 1965. Intracellular distribution and characterization of the lipids of *Streptococcus faecalis* (ATCC 9790). Biochemistry 4:296–305.
22. Wells, M. A., and J. C. Dittmer. 1963. The use of sephadex for the removal of non lipid contaminants from lipid extracts. Biochemistry 2:1259–1263.