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Diphosphatidyl Glycerol in *Mycoplasma laidlawii*

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The phospholipids of *Mycoplasma laidlawii* strain B consist of phosphatidyl glucose and an unidentified compound. This unidentified phospholipid (fraction H) was shown to be neither a nitrogen-containing compound, a plasmalogen, nor a phosphatidyl inositide (Smith and Henrikson, J. Lipid Res. **6**:106, 1965). Further analysis of purified fraction H has led to its identification as a diphosphatidyl glycerol.

Approximately 40 mg of fraction H were isolated in pure form and analyzed by methods previously described (Smith and Henrikson, J. Lipid Res. **6**:106, 1965). Exceptions to these procedures were: (i) the use of chloroform-methanol, 5:1 (v/v), rather than 4:1 (v/v), as eluting solvent for purification by repeated chromatography on silicic acid columns; (ii) the determination of fatty acid esters by the method of Rapport and Alonzo (J. Biol. Chem. **217**:193, 1955); (iii) determination of fatty acids by gas-liquid chromatography of the methyl esters in a Beckman GC2A instrument equipped with a Thermotrac temperature programmer, a thermistor detector, and matched 6-ft (182.9 cm) columns of 20% diethylene glycol succinate on chromosorb W 42/60 under the following conditions: linear temperature program, 100 to 180 C in 15 min; gas, helium; flow rate, 85 ml/min; current, 250 ma; sensitivity, 1, 2, or 5. Fatty acids were identified on the basis of retention times compared with standards (Applied Science Laboratories, State College, Pa.).

TABLE 1. *Analysis of phospholipid fraction H from Mycoplasma laidlawii B**

Fraction	Fatty acid ester		Phosphorus		Glycerol	
	Amt	Ratio	Amt	Ratio	Amt	Ratio
	μmoles		*μmoles*		*μmoles*	
Intact	11.01 ± 0.34	2.05	5.36 ± 0.77	1.00	8.63 ± 0.23	1.61
Diester	—	—	0.26 ± 0.05	1.00	0.38 ± 0.05	1.46

* Values are means of 4 to 10 determinations.

TABLE 2. *Fatty acid composition of phospholipid fraction H from Mycoplasma laidlawii B*

Fatty acid	Per cent total fatty acid
10:0	2.8
—*	0.4
11:0	0.5
12:0	1.6
—	0.2
13:0	0.6
—	2.2
14:0	31.5
15:0	0.2
16:0	54.3
16:1	2.9
17:0	0.2
18:0	0.9
18:1	1.3
—	0.4

* Unidentified.

Table 1 presents the analytical data on the intact phospholipid and product of mild deacylation of fraction H. The molar ratio of phosphorus–glycerol–fatty acid ester of 2:3:4 is compatible with the structure of a diphosphatidyl glycerol. Chromatography of the phosphate diesters of fraction H and known cardiolipin (Difco) in butanol—propionic acid–water (142:71:100, v/v; Benson and Maruo, Biochim. Biophys. Acta **27**: 189, 1958) gave identical R_F values of 0.07, the same as reported for glycerophosphoryl glycerophosphoryl glycerol (Benson and Strickland, Biochim. Biophys. Acta **41**:328, 1960).

The fatty acid composition of fraction H is given in Table 2. Myristic and palmitic acids account for 85% of the total fatty acids. Hydrogenation of the methyl esters in isopropyl alcohol for 12 hr with Adam's catalyst resulted in the disappearance of the 16:1 and 18:1 peaks. The

significance of the types of fatty acids found in the phospholipids of this organism cannot be assessed, because they are similar to the free fatty acids of the culture medium. The extraction procedure for the culture medium does not remove the free fatty acids. The organisms are selective, however, for myristic and palmitic acids.

An effort was made to determine whether phospholipids other than phosphatidyl glucose and diphosphatidyl glycerol were present. Wet, packed cells (about 2 g, dry wt) were extracted after treatment with 5% trichloroacetic acid by the method of Kanfer and Kennedy (J. Biol. Chem. **238**:2919, 1963). No ninhydrin-reacting phospholipids were detectable on thin-layer chromatographs of these extracts, nor were any amino compounds detected in acid-hydrolyzed phospholipid fractions by use of paper chromatography in aqueous phenol of the desalted hydrolysates.

The entire phospholipid content of *M. laidlawii* B appears to be composed of a mixture of phosphatidyl glucose and diphosphatidyl glycerol in a ratio of about 2:1.

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