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Chemical composition of a lipopolysaccharide from *Legionella pneumophila*

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Abstract. Lipopolysaccharide isolated from *Legionella pneumophila* (Phil. 1) was examined for chemical composition. The polysaccharide split off by mild acid hydrolysis contained rhamnose, mannose, glucose, quinovosamine, glucosamine and 2-keto-3-deoxyoctonate, in molar proportions 1.6:1.8:1.0:1.5:4.1:2.7. Heptoses were absent and glucose was probably mainly phosphorylated. The carbohydrate backbone of the lipid A part consisted of glucosamine, quinovosamine and glycerol, in the molar ratios 3.9:1.0:3.4, with glycerol as a phosphorylated moiety. A complex fatty acid substitution pattern comprising eight *O*-ester-linked, exclusively nonhydroxylated acids, and nineteen amide-linked, exclusively 3-hydroxylated acids was revealed. Both straight- and branched (iso and anteiso) carbon chains occurred. The major hydroxy fatty acid was 3-hydroxy-12-methyltridecanoic acid and six others were of a chain-length above 20 carbon atoms, with 3-hydroxy-20-methyldocosanoic acid as the longest. Two dihydroxy fatty acids, 2,3-dihydroxy-12-methyltridecanoic and 2,3-dihydroxytetradecanoic acids, were also detected. These results suggest that *L. pneumophila* contains a rather complex and unusual lipopolysaccharide structure of considerable biological and chemotaxonomic interest.

Key words: *Legionella pneumophila* – Lipopolysaccharide – Lipid A structure – Quinovosamine – 2,3-Dihydroxy-12-methyltridecanoic acid – 3-Hydroxy-12-methyltridecanoic acid

The chemical composition of lipopolysaccharides (LPS) of Gram-negative bacteria has been shown to be useful in taxonomic studies (Mayer 1984; Jantzen and Bryn 1985; Weckesser and Mayer 1988; Wilkinson 1988). Most commonly these macromolecules consist of a lipid A part (a fatty acid substituted glucosamine disaccharide) linked to the polysaccharide part by the rare sugar 2-keto-3-deoxy-D-*manno*-octulosonic acid (KDO). However, the individual monomeric units and types of linkage vary considerably between taxa.

Legionellae are water-borne Gram-negative bacilli which are distinguished from other obligately aerobic, asaccharolytic bacteria by their dependence on L-cysteine for primary isolation (Brenner et al. 1984; Edelstein 1987; Wilkinson 1987) and by unique cellular fatty acids (Mayberry 1981, 1984) and ubiquinones (Collins and Gilbart 1983). *Legionella pneumophila* serogroup 1 was responsible for the Philadelphia epidemic of Legionnaires' disease in 1976, and is still the dominating agent among clinical isolates of *Legionella* (Edelstein 1987).

As measured by DNA-DNA hybridization the relatedness between species of *Legionella* is generally much lower than common for a bacterial genus (Brenner et al. 1984). Furthermore, the taxonomic position of the genus is uncertain in the sense that their DNAs are unrelated to the DNAs of all other species tested (Brenner et al. 1981). Also through oligonucleotide analysis of 16S ribosomal RNA, the legionellae form a distinct subgroup well separated from the other Gram-negative organism tested (Ludwig and Stackebrandt 1983).

In this context it was considered interesting to investigate if this taxonomic uniqueness is reflected in the structure of LPS. Previous studies (Wong et al. 1979; Otten et al. 1986; Friedman et al. 1987; Guerrant et al. 1987), indicate that the polysaccharide part contains KDO and an unusual 2-aminodideoxyhexose. No structural information is available for the lipid A part of the LPS except that it is easily cleaved off upon hydrolysis. However, analysis of cellular fatty acids generally reveals a remarkably complex and unusual pattern of amide-linked 3-hydroxy fatty acids, which most likely originate from LPS (Mayberry 1981, 1984; Wilkinson et al. 1987). A few species, including the two most common pathogenic species *L. pneumophila* and *L. micdadei* also contain di-hydroxylated acids (Mayberry 1984).

In this communication we report on the chemical composition of an LPS preparation from *L. pneumophila*. It will be shown that the composition, especially of the lipid A part, differs considerably from LPS structures of most other Gram-negative bacteria.

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Abbreviations: LPS, lipopolysaccharide; PS, polysaccharide; KDO, 2-keto-3-deoxy-octonate; GC, gas chromatography; GC-MS, gas chromatograph-mass spectrometer combined instrument; CI, chemical ionization; EI, electron impact; HF, hydrofluoric acid; TFA, trifluoroacetyl; TMS, trimethylsilyl

Materials and methods

Bacterial strains and cultivation. The Phil.1 strain of *L. pneumophila*, originally obtained from R. Weaver, Centers for Disease Control, Atlanta, USA, was grown (2 days, 37°C) on buffered charcoal yeast extract (BCYE agar, Oxoid, Basingstoke, GB) (Edelstein 1982). Cell material from 100 agar plates was harvested with distilled water, centrifuged (Sorvall RC-5B, 10,000 × *g*, 20°C), washed once with distilled water, killed by heating at 65°C for 60 min in a water-bath and freeze-dried.

Isolation of LPS. LPS was isolated from lyophilized bacterial cells using largely the methods of Darveau and Hancock (1983) and Galanos et al. (1969). Dried bacterial cells (1 g) were suspended in 30 ml of 10 mM Tris-HCl (pH 8.0) containing 2 mM $MgCl_2$, 3 mg of pancreatic DNase I (Sigma Chemical Company, St. Louis, MO, USA) and 750 µg of pancreatic RNase A (Sigma). The suspension was sonicated with five 30 s bursts at max. probe energy (Branson sonifier B 12, Danbury, Connecticut, USA) whereafter additional DNase and RNase were added to final concentrations of 200 and 50 µg/ml, respectively. After incubation at 37°C for 2 h, tetrasodium EDTA (Sigma) and sodium dodecyl sulfate (SDS) solutions were added yielding a final volume of 50 ml, containing 0.1 M EDTA, 2% SDS and 10 mM Tris-HCl (pH 9.5). Peptidoglycan was removed by centrifugation at 50,000 × *g* for 30 min at 20°C. Pronase (Sigma) was added to the supernatant to a concentration of 200 µg/ml and the solution was incubated overnight at 37°C with constant agitation. Crude LPS was precipitated at 0°C by adding two volumes of 0.375 M $MgCl_2$ in 95% ethanol, and isolated by centrifugation at 12,000 × *g* for 15 min at 0°C. The pellet was dissolved in 25 ml of 10 mM Tris-HCl (pH 8.0) containing 0.1 M EDTA and 2% SDS, and sonicated twice at maximum probe energy. The pH was lowered to 7 by dropwise addition of 4 M HCl, and the sample was incubated at 85°C for 30 min. After cooling to room temperature, the pH was raised to 9.5 with 4 M NaOH, pronase was added to 25 µg/ml, and the solution was agitated overnight at 37°C. LPS was precipitated at 0°C with 2 volumes of 0.375 M $MgCl_2$ in 95% ethanol and isolated by centrifugation as above. The pellet was resuspended in 20 ml of 10 mM Tris-HCl (pH 8.0), sonicated, dialyzed for 48 h against water and lyophilized. The freeze-dried LPS preparation was extracted three times with 8 ml of PCP-solution (phenol:chloroform:hexane, 2:5:8) and centrifuged at 2,000 × *g*; the pooled supernatants were evaporated on a rotary evaporator. Water was added to the residual phenol solution, but as no precipitation occurred, phenol was removed through dialysis against water for 48 h and the LPS was obtained after lyophilization.

Cleavage of LPS into lipid A and polysaccharide. LPS was cleaved by 0.1 M acetate buffer (pH 4.4) at 100°C for 1 h (Brade and Brade 1985). The precipitated lipid A was isolated by centrifugation at 1,000 × *g*, extracted twice with water and once with acetone, and lyophilized. The polysaccharide (PS) was obtained by freeze-drying of the acetate solution.

Periodate oxidation of LPS was performed as described by Pazur (1986).

Quantitative analyses by GC. Neutral and aminosugars were analysed both as alditol acetates (Sawardeker et al. 1965) and as trifluoroacetylated (TFA) methylglycosides (Bryn and Jantzen 1982, 1986). For determination as alditol acetates, neutral sugars were liberated by hydrolysis in 0.1 M HCl at 100°C for 48 h (Yokata et al. 1987), and arabinitol was used as internal standard. KDO was determined as TFA methylglycosides after methanolysis (2 M HCl, 60°C, 2 h) followed by TFA-derivatization as described (Bryn and Jantzen 1986). Aminosugars were determined both as alditol acetates after hydrolysis in 4 M aqueous HCl, 100°C, 18 h (Yokata et al. 1987), and as TFA methylglycosides after methanolysis in 4 M methanolic HCl, 100°C, 18 h. The TFA-methylglycosides were quantified using molar response factors determined relative to TFA-derivatized 3-hydroxymyristic acid (Bryn and Jantzen 1982). Phosphorylated sugars were quantified by measuring differences before and after dephosphorylation of LPS by aqueous 48% (w/w) HF at 4°C for 72 h (Sonesson et al. 1989).

Total fatty acids were determined by GC after release by 4 M HCl in methanol, 100°C, 18 h followed by trifluoroacetylation and with n-nonadecanoic acid as internal standard. Ester-linked fatty acids were determined after liberation by 2 M HCl at 60°C for 2 h (Bryn and Jantzen 1986). Amide-linked acids were calculated by difference. GC analyses were performed using one DANI 6500-HR (Monza, Italy) and one Carlo Erba Model 4160 instrument (Rodano, Italy), both equipped with flame-ionization detectors. Two fused-silica capillary columns were used; one (25 m × 0.2 mm) with cross-linked SE-30 as liquid phase (SGE, Ringwood, Victoria, Australia) and one (30 m × 0.3 mm) with cross-linked SE-52 (J&W, Folsom, CA, USA).

GC-MS. The solutions of TFA methylglycosides, alditol acetates and fatty acid methyl esters (TFA or TMS derivatized), made for GC analysis as described above, were generally also subjected to GC-MS analysis. Sugar phosphates and glycerophosphate were liberated and TMS-derivatized as described (Harvey and Horning 1973; Zamze et al. 1987). GC-MS analyses were carried out using both a Finnigan Ion-Trap 700 (San Jose, CA, USA) connected to a Varian 3300 GC (Walnut Creek, CA, USA) with a (25 m × 0.2 mm) fused-silica SE-30 column (Hewlett-Packard, Avondale, PA, USA), and a Ribermag R10-10c quadropole (Rueil-Malmaison, France) in connection with a Carlo Erba GC (as above) with a (25 m × 0.2 mm) fused-silica SE-54 column (SGE). Both instruments were used with electron-impact ionization (EI) at 70 eV ion energy in the mass range 50 to 650. In addition the Ribermag MS was also used in the chemical ionization (CI) mode with ammonia as the reagent gas (at 0.07 torr) ionized with electrons at an energy of 93 eV.

Identification of fatty acids and sugars. In addition to the use of retention time data, the fatty acid methyl esters were also identified by their MS spectra; the hydroxylated both as TFA- and TMS derivatives (Mayberry 1981, 1984). GC-MS identification of neutral sugars and aminosugars was performed as alditol acetates (Lönngren and Svensson 1974) and as TFA-methylglycosides (König et al. 1973). KDO was identified by GC-MS as previously described (Bryn and Jantzen 1986). Sugar phosphates and glycerol phosphates were released by 4 M aqueous HCl, TMS-derivatized, and GC-MS analyzed as described (Harvey and Horning 1973; Zamze et al. 1987).

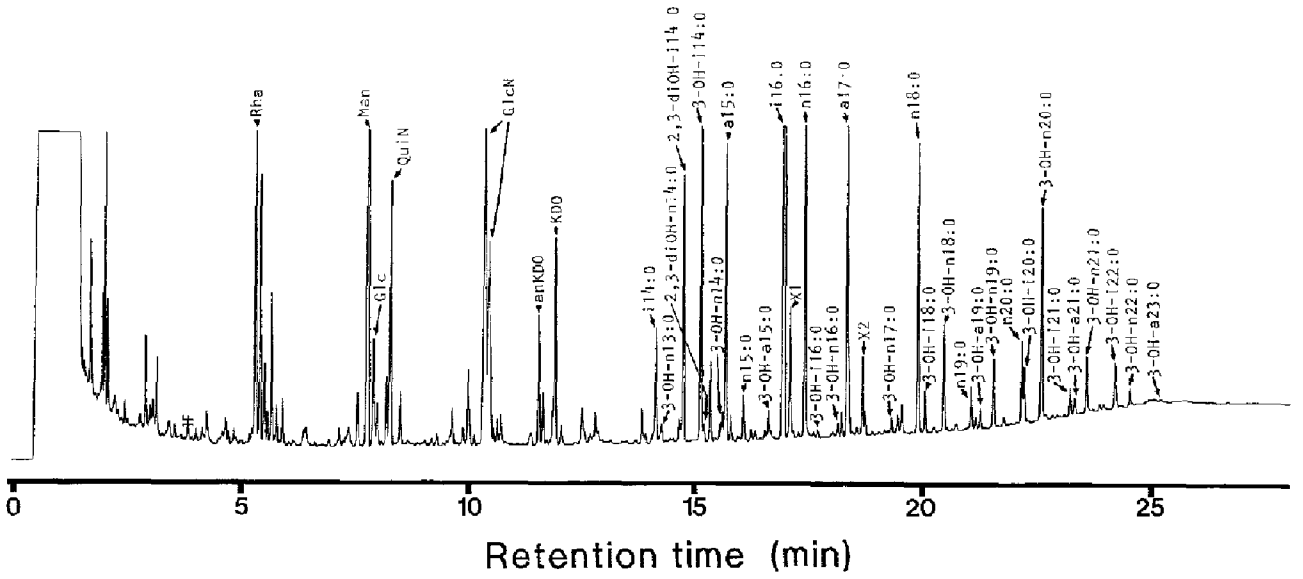

Fig. 1. Gas chromatogram of *L. pneumophila* (Phil. 1) LPS after methanolysis (2 M hydrochloric acid in methanol, 85°C, 18 h), trifluoroacetylation and analysis on a 25 m (0.22 mm I.D.) SE-30 fused-silica capillary column. Temperature programmed from 90 to 280°C at 8°C/min. *Abbreviations:* see Table 1, and in addition; X1, X2, unknown compounds of polar nature; anKDO, anhydro form of TFA methylglycoside derivative of KDO (Bryn and Jantzen 1986); n19:0, nonadecanoic acid (internal standard)

Chemicals. Solvents were of p.a. quality. Distilled water was Millipore (Molsheim, France) purified, D-Glucose-6-phosphate, L-α-glycerophosphate, and β-glycerophosphate were from Sigma. Chemically synthetized alditol acetates of 2-amino-2,6-dideoxy-D-glucose (quinovosamine), 2-amino-2,6-dideoxy-D-galactose (fucosamine) and 2-amino-2,6-dideoxy-L-talose (pneumosamine) were kindly provided by Pia Seffers, University of Stockholm, Sweden. Chemically synthesized methyl-α-L-fucosamine was a gift from Ulf Lindquist, The National Bacteriology Laboratory, Solna, Sweden.

Results

Isolation of LPS

The "cold ethanol extraction" procedure by Darveau and Hancock (1983) was applied, previously reported to be efficient for the isolation of *Legionella pneumophila* LPS (Gabay and Horwitz 1985; Ciesielski et al. 1986). Ribose was found in this crude LPS, indicating contamination with nucleic acids, and a PCP-extraction step (Galanos et al. 1969) was added to the purification procedure. The final product contained negligible amounts of nucleic acids. Contamination of phospholipids was not apparent since the monounsaturated C_{15}, and C_{16} fatty acids found in extractable lipids (Mayberry 1981, 1984), were absent. The final yields varied between 1–4% wt (based on dried bacteria) for three different batches.

Chemical analysis of LPS

A gas chromatogram showing the carbohydrate and fatty acid constituents of *L. pneumophila* LPS is presented in Fig. 1. The main neutral sugars were rhamnose, mannose and glucose (Table 1). The latter sugar may occur mainly in a phosphorylated state as hydrofluoric acid treatment increased the yield of glucose from 10 to 44 pmol/μg LPS. Similarly glycerol was also present probably largely in a phosphorylated state (see below). The two aminosugars, glucosamine (6.9% wt) and quinovosamine (1.9% wt), as well as KDO (2.8% wt), were most likely unphosphorylated since HF treatment gave no change in the yield of these constituents.

Sugar constituents were identified by their retention times and MS fragmentation both as alditol acetates and TFA-methylglycosides. Alditol acetates were also analysed by GC-MS in the CI mode. The significant fragment ions of TFA-methylglycoside of KDO (as methyl ester), *m/z* 591, 477 and 363 (Bryn and Jantzen 1986), confirmed the identity of this typical LPS constituent.

The primary indication of glycerophosphate being an LPS constituent was based on the increased amount of glycerol obtained by HF-dephosphorylation. This observation was confirmed by comparison with the authentic L-α-glycerophosphate and β-glycerophosphate. These two compounds were analysed by GC-MS as TMS-derivatives according to Harvey and Horning (1973), and both retention times and the characteristic MS fragmentation patterns were identical for two abundant peaks from *L. pneumophila* LPS obtained after hydrolysis, fractionation on an ion-exchange column and TMS-derivatization (Zamze et al. 1987). Due to phosphate migration both the α- and β-isomer were formed during the hydrolytic process (Harvey and Horning 1973).

The occurrence of a glucose-phosphate constituent was also indicated by the HF-procedure (Table 1). However, the attemps at a verification by GC-MS was largely inconclusive due to the lack of appropriate reference compounds.

As seen in Table 1 the sugars in *L. pneumophila* LPS were present in the relative molar proportions: glucose, 1.0; mannose, 1.8; rhamnose, 1.6; KDO, 2.7; quinovosamine, 2.7; glucosamine, 8.8; glycerol, 7.7 and the total amount of carbohydrate was 18.1% wt.

The complex fatty acid composition (Table 1) revealed the presence of eight different straight or branched (iso and

Table 1. Chemical composition of LPS from *Legionella pneumophila* (Phil. 1)[a]

Component	LPS		Lipid A[b]	PS[c]
	pmol/µg	mol %	pmol/µg LPS	pmol/µg LPS
GlcN	386	20.5	209	268
QuiN	118	6.3	54	109
KDO	119	6.3	–	88
Rha	69	3.7	–	56
Man	78	4.1	–	78
Glc	10 (44)[d]	(2.3)	–	12
Glycerol-P	(340)	(18.0)	(181)	–
i14:0	33.5	1.8	7.3	
a15:0	53.9	2.9	31.6	
n15:0	3.2	0.2	4.9	
i16:0	210.3	11.2	154.0	
n16:0	24.6	1.3	42.1	
a17:0	32.9	1.7	39.8	
n18:0	17.2	0.9	35.0	
n20:0	6.7	0.4	14.8	
3-OH-n13:0	3.4	0.2	3.6	
3-OH-i14:0	81.1	4.3	81.6	
3-OH-n14:0	5.2	0.3	5.2	
3-OH-a15:0	12.6	0.7	13.0	
3-OH-i16:0	4.3	0.2	4.1	
3-OH-n16:0	5.2	0.3	5.3	
3-OH-n17:0	1.7	0.1	1.6	
3-OH-i18:0	6.2	0.3	5.7	
3-OH-n18:0	15.6	0.8	15.1	
3-OH-a19:0	5.0	0.3	5.0	
3-OH-n19:0	12.1	0.6	12.3	
3-OH-i20:0	12.3	0.6	11.1	
3-OH-n20:0	63.6	3.4	66.0	
3-OH-i21:0	4.9	0.3	5.5	
3-OH-a21:0	9.8	0.5	9.5	
3-OH-n21:0	17.6	0.9	18.3	
3-OH-i22:0	12.4	0.7	12.8	
3-OH-n22:0	11.9	0.6	11.5	
3-OH-a23:0	2.3	0.1	2.0	
2,3-diOH-i14:0	56.2	3.0	57.9	
2,3-diOH-n14:0	4.2	0.2	4.7	

Abbreviations: GlcN, glucosamine; QuiN, quinovosamine; KDO, 2-keto-3-deoxy-octonate; Rha, rhamnose; Man, mannose; Glc, glucose; i14:0, 12-methyltridecanoic acid; a15:0, 12-methyltetradecanoic acid; n15:0, pentadecanoic acid; i16:0, 14-methylpentadecanoic acid; n16:0, hexadecanoic acid; a17:0, 14-methylhexadecanoic acid; n18:0, octadecanoic acid; n20:0, eicosanoic acid; 3-OH-n13:0, 3-hydroxytridecanoic acid; 3-OH-i14:0, 3-hydroxy-12-methyltridecanoic acid; 3-OH-n14:0, 3-hydroxytetradecanoic acid; 3-OH-a15:0, 3-hydroxy-12-methyltetradecanoic acid; 3-OH-i16:0, 3-hydroxy-14-methylpentadecanoic acid; 3-OH-n16:0, 3-hydroxyhexadecanoic acid; 3-OH-n17:0, 3-hydroxyheptadecanoic acid; 3-OH-i18:0, 3-hydroxy-16-methylheptadecanoic acid; 3-OH-n18:0, 3-hydroxyoctadecanoic acid; 3-OH-a19:0, 3-hydroxy-16-methyloctadecanoic acid; 3-OH-n19:0, 3-hydroxynonadecanoic acid; 3-OH-i20:0, 3-hydroxy-18-methylnonadecanoic acid; 3-OH-n20:0, 3-hydroxyeicosanoic acid; 3-OH-i21:0, 3-hydroxy-19-methyleicosanoic acid; 3-OH-a21:0, 3-hydroxy-18-methyleicosanoic acid; 3-OH-n21:0, 3-hydroxy-uncosanoic acid; 3-OH-i22:0, 3-hydroxy-20-methyluncosanoic acid; 3-OH-n22:0, 3-hydroxydocosanoic acid; 3-OH-a23:0, 3-hydroxy-20-methyldocosanoic acid, 2,3-diOH-i14:0, 2,3-dihydroxy-12-methyltridecanoic acid; 2,3-diOH-n14:0, 2,3-dihydroxytetradecanoic acid

[a] Carbohydrates and fatty acids were determined as TFA-methylglycosides and methyl esters (TFA-derivatized), respectively, except GlcN, QuiN and glycerol which were determined as alditol acetates

[b] Relative values were normalized to LPS values by the use of total amounts of hydroxy fatty acids (see text)

[c] Relative values were normalized to LPS values by the use of amounts of mannose (see text)

[d] Values in parentheses were obtained for hydrofluoric acid treatment, other values for the native products

anteiso) nonhyroxy fatty acids, nineteen 3-hydroxy fatty acids and two dihydroxy fatty acids. The major nonhydroxy fatty acid was 14-methylpentadecanoic acid; all eight acids of this type were exclusively ester-bound as they were released by mild methanolysis (2 M HCl, 60°C, 2 h). On the other hand, the hydroxylated fatty acids were all bound in stable, presumably amide-linkages. The most prominent 3-hydroxy fatty acid was 3-hydroxy-12-methyltridecanoic acid.

The identities of all fatty acids were established by GC retention-time and MS (EI) data (Mayberry 1981, 1984). Hydroxylated fatty acid methyl esters were analysed both after trifluoroacetylation and trimethylsilyl derivatization. The trimethylsilylated dihydroxy fatty acid methyl esters

showed the significant fragment ions of *m/z* 257 and 234 as reported by Mayberry (1981).

The molar distribution of the fatty acid classes was 52% nonhydroxy acids, 39% monohydroxy acids and 8% dihydroxy acids. On a weight basis 9.7% nonhydroxy fatty acids, 8.6% monohydroxy fatty acids and 1.6% dihydroxy fatty acids were present in the LPS.

Chemical analysis of PS and lipid A

Mild acid hydrolysis of LPS gave a pellet, lipid A, and the so-called "degraded polysaccharide" (PS) which both were analysed by GC. The PS showed only traces of fatty acids and lipid A was completely devoid of KDO, rhamnose, mannose and glucose, demonstrating a negligible cross-contamination between the PS and lipid A fractions.

A constant ratio (coefficient of variation: 6%) between the lipid A and LPS hydroxy fatty acid values, was found. This allowed us to transform the data from the lipid A analyses into LPS values. Thus, for each LPS component we could calculate the contribution from lipid A (Table 1). Similarly, the mannose values were used to determine the contribution from PS. The given PS values are, however, less accurate probably mainly due to an unspecific loss of mannose in the analytical assay. As seen in Table 1, this inconstancy is also reflected in the inaccurate "total recovery" of sugars when adding the lipid A and PS values.

Carbohydrates found in the lipid A precipitate were glucosamine, quinovosamine and glycerol, the latter apparently as the sole phosphorylated entity (Table 1). The fatty acids were the same as in native LPS, but the relative amounts were slightly different, indicating that some ester linked nonhydroxy fatty acids were lost during isolation of lipid A. Nonhydroxy acids constituted the major fatty acid class (48 mol%) followed by monohydroxy acids (43 mol%) and dihydroxy acids (9 mol%). By weight the lipid A consisted of 20% carbohydrates, 28% nonhydroxy fatty acids, 30% monohydroxy fatty acids and 5.2% dihydroxy fatty acids.

Discussion

The most notable taxonomic feature of the *L. pneumophila* LPS is probably related to the lipid A structure. A pattern of nineteen 3-hydroxy fatty acids in the 13 – 23 carbon atom range, two dihydroxy fatty acids, and eight ester-linked nonhydroxylated fatty acids, is a remarkably complex and distinct characteristic of LPS from *L. pneumophila*. This complicated fatty acid substitution of lipid A is most likely a common feature of the presently recognized *Legionella* species. Their individual cellular fatty acid composition have been recorded and shown to contain a comparably complex and distinct pattern of hydroxylated fatty acids, all presumably of LPS origin (Mayberry 1981, 1984; Wilkinson et al. 1987).

As can be calculated from Table 1, the aminosugars, hydroxy fatty acids, and unhydroxylated fatty acids occur in approximately equimolar proportions (14.0, 15.4 and 17.5, respectively). These figures are consistent with the general lipid A model having a glucosamine disaccharide with 3-hydroxy fatty acids in amide-linkages, as a central structural unit.

A few other taxa have been shown to possess a lipid A fatty acid pattern different to that of *L. pneumophila* but of an almost comparable complexity. Thus the rickettsial agent of Q fever, *Coxiella burnetii*, contains 24 different acyl residues of which nine are 3-hydroxy fatty acids (methyl-branched or non-branched) in the 12 – 18 carbon atom range (Wollenweber et al. 1985). The lipid A fatty acid pattern of the pseudomonad-like bacterium *Alteromonas putrefaciens* (or *Shewanella putrefaciens*) was found to contain eleven 3-hydroxy fatty acids (methyl-branched and non-branched) in the 11 – 15 carbon atom range (Moule and Wilkinson 1989). A pattern of 12 fatty acids including a 3-hydroxy fatty acid of 22 carbon atoms has been found in lipid A of *Chlamydia trachomatis* (Nurminen et al. 1985), but a 3-hydroxy-fatty acid of as many as 23 carbon atoms has not to our knowledge been reported previously.

The carbohydrate backbone structure of lipid A also deviates from most lipid A structures by the presence of quinovosamine and glycerophosphate. Quinovosamine has previously tentatively been identified as a lipid A (and PS) constituent in LPS from the purple sulfur bacterium *Thiocystis violacea* (Meissner et al. 1988).

Some structural information concerning quinovosamine has been obtained by periodate oxidation, where the sugar appeared to be protected (10% loss). This indicates that quinovosamine, if present as a hydrophilic extension of the glucosamine disaccharide backbone, or as a polar head group, must be at least partly acylated. Further experimental data, e.g. from methylation studies and NMR, are required for obtaining a more complete picture of the structural position of this constituent.

Since HF treatment did not render more of the two aminosugars of lipid A detectable by GC, they are probably unphosphorylated. Corresponding deviation from the enteric lipid A model by lack of phosphate substitution has also been found for lipid A from several other taxa (Weckesser and Mayer 1988), including the purple sulfur bacteria (*Chromatiaceae*) representing the gamma-1 branch of the phylogenetic tree based on 16S rRNA catalogues (Woese et al. 1985). Our results thus indicate that the gamma-2 branch of this tree, which currently includes *Legionella* species as the single taxonomic entity, may share this lipid A structural characteristic.

Atypical as a lipid A constituent is also glycerophosphate, which to our knowledge previously has only been described as a lipid A constituent in *Haemophilus influenzae* (Zamze et al. 1987). It is unlikely that the origin of this constituent was phospholipid impurities as the unsaturated C_{15} and C_{16} fatty acids found in extractable lipids (Mayberry 1984; unpublished results) were absent in the LPS preparation. About 50% loss of glycerol (phosphorylated) from lipid A was observed after the mild hydrolytic cleavage of LPS. This may indicate that glycerophosphate is bound in an acid labile linkage, e.g. in the C1 position of a glucosamine disaccharide. Structural analysis of intact lipid A backbone is in progress.

The PS part appears to be of an unusually short length, as indicated by a sugar/fatty acid ratio of 0.75. This can be compared with corresponding values for LPS of *Escherichia coli* 09 (12.4), *S. abortus equi* (8.4), and *Neisseria meningitidis* (2.2) (Wedege et al. 1988). PS contained the common LPS sugars rhamnose, mannose, glucose, glucosamine and KDO as reported by Otten et al. (1986). In addition we found the more uncommon aminosugar quinovosamine, previously found in LPS of *Vibrio cholerae* 0:2 (Kenne et al. 1988) and in LPS of two strains of *A. putrefaciens* (Moule and

Wilkinson 1989). L-Glycero-D-mannoheptose, a common constituent of the LPS core, was not found in the *Legionella* LPS examined by us. This sugar often occurs masked in a phosphorylated form, but repeated analysis after dephosphorylation by HF treatment was also negative for this sugar. The amount of glucose, however, increased fourfold after dephosphorylation. Mannitol, reported by Guerrant et al. (1987) to be an LPS constituent of *L. pneumophila*, was not found by us.

It has been reported that *L. pneumophila* LPS is far less toxic in mice (1–3%) and has low pyrogenic response (rabbits) when compared to *Salmonella* LPS (Wong et al. 1979). This is in agreement with our observation that it structurally deviates from the established LPS model of *Enterobacteriaceae*. The two terminal phosphate groups bound to the glucosamine disaccharide backbone of the latter lipid A structure, essential for the complete array of endotoxic effects (Rietschel et al. 1987), was not found in the *L. pneumophila* lipid A.

The uniqueness of *L. pneumophila* LPS was also reflected in the banding pattern obtained by sodium dodecyl sulfate polyacrylamide electrophoresis which differed substantially from other Gram-negative bacteria (Nolte et al. 1986). This deviation was probably due to the structural uniqueness of the lipid A part which did not co-migrate with a corresponding lipid A preparation of *S. minnesota* (Otten et al. 1986).

Preliminary results on the chemical composition of LPS from other *Legionella* species indicate a similar complex structure. Both phosphorylated glucose and glycerophosphate were found for LPS of *L. micdadei* LPS (unpublished results), and as mentioned previously the very complex pattern of the typical LPS constituents, 3-hydroxy fatty acids, may be a general characteristic of the legionellae. It appears therefore that LPS may serve as a useful chemotaxonomic hallmark of genus *Legionella*. Further structural studies of LPS from other species representing the different DNA-DNA hybridization groups of *Legionella* may also contribute to a better understanding of the intrageneric relationships between the individual *Legionella* species.

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