Determination of the Structure of the Lipid A Fraction from the Lipopolysaccharide of *Pseudomonas Cichorii* by Means of NMR and MALDI-TOF Mass Spectrometry

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Dedicated to Professor Lorenzo Mangoni on occasion of his 70th birthday

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The chemical structure of lipid A from the lipopolysaccharide of the plant-associated bacterium *Pseudomonas cichorii*, was elucidated by compositional analysis and spectroscopic methods (MALDI-TOF and 2D NMR). The sugar's backbone was constituted by the β-(1′→6)-linked D-glucosamine disaccharide 1-phosphate. The lipid A fraction showed remarkable heterogeneity with respect to the fatty acid and phosphate composition. The major species are hexaacylated and pentaacylated lipid A, bearing the (*R*)-3-hydroxydodecanoic acid [C12:0 (3OH)] in amide linkage and an (*R*)-3-hydroxydecanoic [C10:0 (3OH)] in ester linkage while the secondary fatty acids are present as C12:0 and C12:0 (2-OH). A nonstoichiometric phosphate substitution at position C-4′ of the distal GlcN was detected. Interestingly, the pentaacyl lipid A is lacking a primary fatty acid, namely the C10:0 (3-OH) at position C-3′. A potential biological meaning of this peculiar lipid A is also suggested.

Introduction

Lipopolysaccharides (LPS) are complex macromolecules and are the main component of the outer membrane of Gram-negative bacteria. They are built up of three covalently linked regions, genetically and structurally distinct: the *O*-specific polysaccharide (O-chain, O-antigen), the core oligosaccharide and a glycolipid portion, termed lipid A, which anchors the molecule to the outer layer of bacterial outer membrane.

It is nowadays established that lipid A constitutes the endotoxic principle of the LPS molecule, expressing all the pathophysiological effects known to be induced by the LPS molecule.[1] Therefore, the study and the structural elucidation of these complex glycolipids is of high importance.

In animal-associated bacteria, lipid A has a rather conservative structure usually consisting of a glucosamine (GlcN) disaccharide backbone which is phosphorylated at positions 1 and 4′ and is acylated with primary 3-hydroxy fatty acids at the positions 2, 3, 2′, and 3′ of the GlcN I (proximal) and GlcN II (distal) residues.[1] The primary fatty acids, in turn, can be acylated by other fatty acids (secondary fatty acids).

The achievement of intact lipid A preparation is possible because of the acid-labile ketosidic linkage between core oligosaccharide and lipid A. This linkage is between the Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid) and the distal glucosamine (GlcN II).

To date, very little is known about the structure and functions of lipid A in phytopathogenic associated bacteria,[2] yet it should be important in understanding of mechanisms of infection.

Moreover, the study of lipid A structures from Gram-negative bacteria is particularly important in order to identify lipid A analogues which can antagonise the biological activation of competent mammalian host cells by lipid A. This has been the case of the lipid A of *Rhodobacter capsulatus* and its synthesised analogue labelled as E5531.[3]

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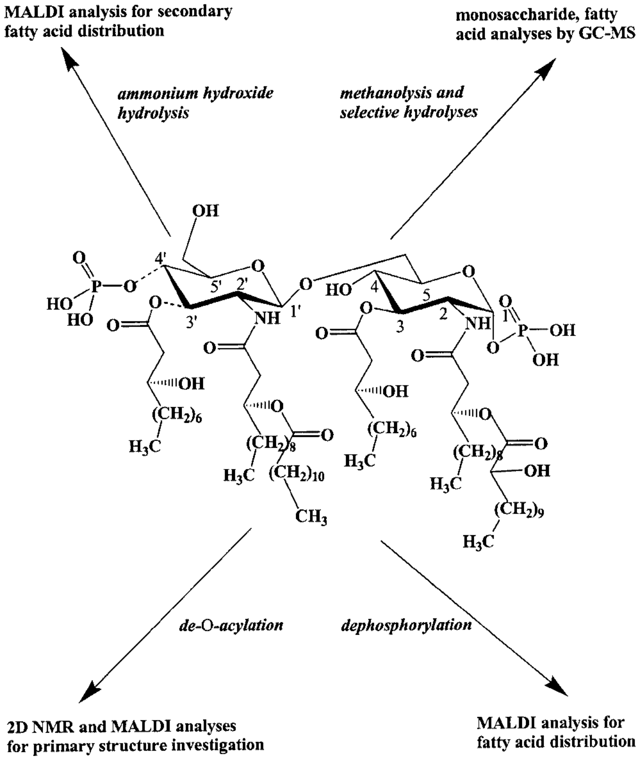

Scheme 1

The lipid A fraction of the bacterium *Pseudomonas cichorii* was analysed within this frame.

Pseudomonas cichorii is a Gram-negative bacterium which on lettuce causes a disease known as "varnish spot"[4] and was originally isolated from cichorii and subsequently reported from many other hosts such as cabbage, tomato, celery, tobacco, coffee, and several ornamental plants, grown in open-field. On lettuce the disease is characterised by large necrotic brown-blackish areas that occur on the blades and petioles of leaves. Leaf spots turn to large soft rots with frequent rain or high relative humidity.

Results and Discussions

The extraction of the dried cells according to the hot phenol/water method[5] yielded LPS in the phenol phase. After workup, pure LPS was obtained. The ladder-like pattern of the SDS gel electrophoresis indicated the presence of a polymeric form of LPS (S-form LPS).[6] The pure lipid A fraction was obtained as sediment by mild hydrolysis of the lipopolysaccharide with sodium acetate buffer at pH = 4.4. A series of selective degradations combined with MS and NMR analyses led to define the structure of the lipid A fraction of *P. cichorii* (Scheme 1).

The monosaccharide analysis revealed the presence of terminal nonreducing GlcN and 6-substituted GlcN, both

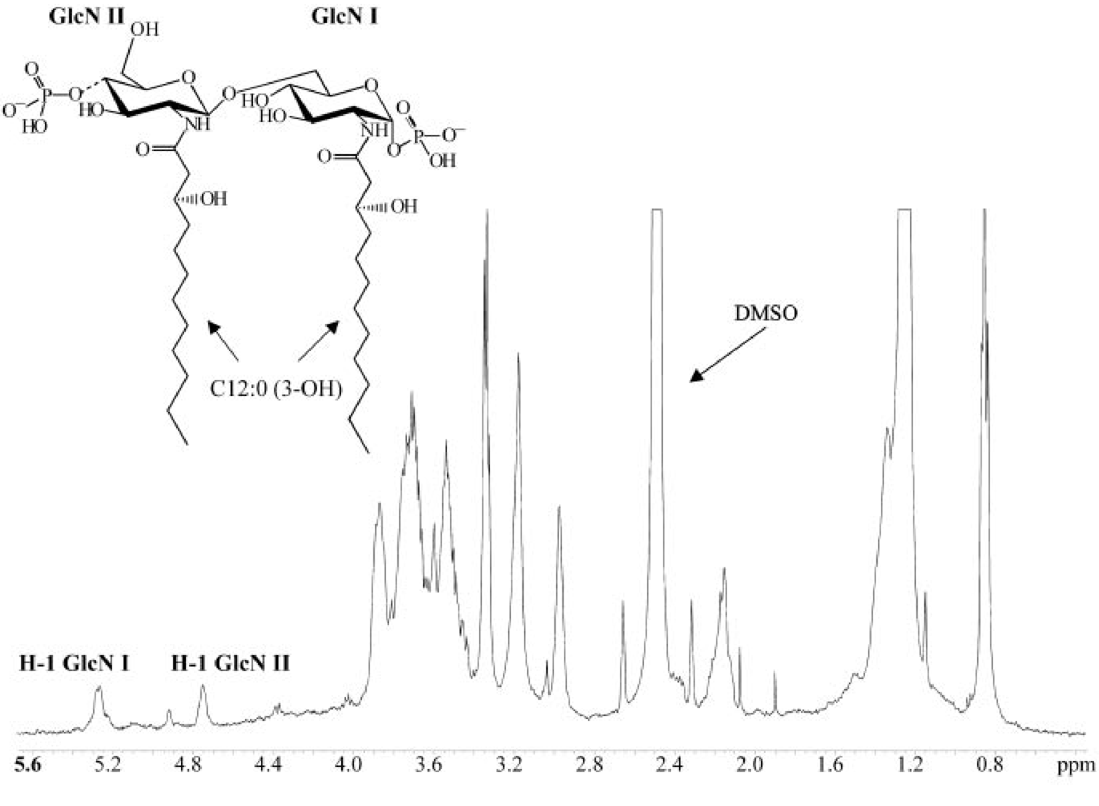

Figure 1. ^{1}H NMR spectrum and the structure of de-*O*-acylated lipid A from *P. cichorii*; the spectrum was measured in [D$_6$]DMSO at 343 K

Table 1. ^{1}H-,^{13}C-, and ^{31}P NMR resonances of the bis(phosphorylated) de-*O*-acylated lipid A disaccharide backbone; two-dimensional spectra were obtained at 343 K in [D$_6$]DMSO and chemical shifts are expressed in δ relative to dimethyl sulfoxide (δ_H = 2.49 ppm, δ_C = 39.7 ppm); the anomeric coupling constants $^{1}J_{C,H}$ and $^{3}J_{H1,H2}$ are given in parentheses

Position	δC	δH	δP
1	92.0	5.27	−1.5
	($^{1}J_{C,H}$ = 173 Hz)	$^{3}J_{H1,H2}$ = 3.3 Hz	
2	54.6	3.66	
3	74.4	3.93	
4	71.4	2.94	
5	71.2	3.52	
6a/6b	67.3	3.87/3.84	
2-NH		7.21	
1′	100.9	4.76	
	($^{1}J_{C,H}$ = 165 Hz)	$^{3}J_{H1,H2}$ = 7.7 Hz	
2′	55.6	3.50	
3′	72.3	3.71	
4′	70.8	3.77	3.0
5′	75.8	3.14	
6′a/6′b	61.0	3.65/3.92	
2′-NH		7.63	

with D-configuration.[7] No other sugars were detected. Phosphate was detected by colorimetric assay.[8]

The total fatty acid content was determined on an aliquot of the sample by means of acid hydrolysis, methylation with diazomethane and GC-MS analysis. In parallel, a different aliquot of the sample was hydrolysed under mild alkaline conditions in order to cleave only the ester linkages and then analysed by GC-MS.[9] A third aliquot of the sample was allowed to react with a chiral reagent and then analysed by GC-MS, this last operation defined the absolute configuration of the fatty acids.[10,11]

These analyses revealed the presence of (*R*)-3-hydroxydodecanoic [C12:0 (3-OH)] in amide linkage and of (*R*)-3-hydroxydecanoic [C10:0 (3-OH)], (*S*)-2-hydroxydecanoic [C12:0 (2-OH)] and dodecanoic acid (C12:0) in ester linkage.

In order to improve the solubility of the product in any solvent a consistent aliquot of the product was de-*O*-acylated. The fatty acid analysis confirmed the presence of (*R*)-3-hydroxydodecanoic [C12:0 (3-OH)] as the only kind of fatty acid amide linked to the GlcN backbone.

Since this product revealed a good solubility in DMSO at 343 K and the ^{1}H NMR spectrum of the product was of good quality (Figure 1), a full 2D NMR analysis was performed (COSY, TOCSY, ROESY, HSQC).

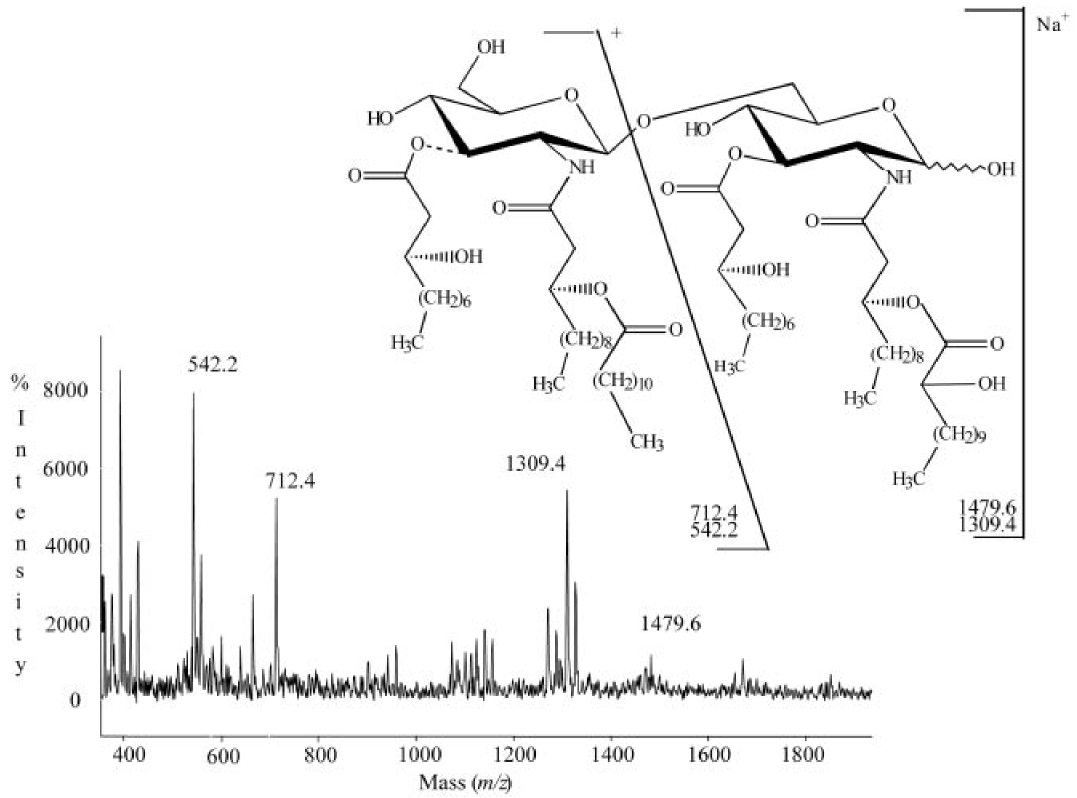

Figure 2. Positive ion MALDI-TOF mass spectrum of dephosphorylated lipid A from *P. cichorii*; the inset is showing the structure of dephosphorylated lipid A and the deriving oxonium ions; the dotted line indicates the nonstoichiometric acylation by C10:0 3-OH fatty acid

The NMR spectroscopic data are in full agreement with the presence of a GlcN disaccharide backbone. Actually, in the ^{1}H,^{13}C HSQC spectrum two ^{1}H anomeric resonances at δ = 5.27 and 4.76 ppm correlate with two signals at δ = 92.1 and 100.9 ppm, respectively. The chemical shifts (Table 1), the $^3J_{H1,H2}$ and the $^1J_{C,H}$ coupling constants are diagnostic of the α- and β-anomeric configurations for GlcN I and GlcN II, respectively. In the ROESY spectrum, concerning GlcN II, the observation of the typical intraresidual ROE effects between H1, H3 and H5 confirms the β-anomeric configuration. Moreover, the anomeric proton of GlcN II shows *inter*-residue cross peaks with the two protons H6a and H6b (δ = 3.87 and 3.84 ppm) and H4 of GlcN I (δ = 2.94 ppm). These data, together with the glycosylation shift (δ = 67.3 ppm) of C6 of GlcN I, prove the β-(1→6) linkage between the two monosaccharides. The phosphate substitution is inferred by a ^{1}H,^{31}P HSQC spectrum which indicates the anomeric substitution of GlcN I and the 4′-substitution of GlcN II.

Therefore, with the above data, the de-*O*-acylated lipid A was demonstrated to be built up of β (1→6)-D-GlcN disaccharide, acylated at the C2 and C2′ positions by 3-hydroxy fatty acids C12:0 (3-OH) and phosphorylated at the C-1 and C4′ positions (Figure 1).

In order to gain further structural information on the fatty acid distribution, an aliquot of lipid A was dephosphorylated with 48% aqueous HF. The positive ion MALDI mass spectrum of the dephosphorylated product (Figure 2) reveals two pseudomolecular ion peaks at *m*/*z* = 1479.6 and 1309.4 $[M + Na]^+$, attributable to hexaacyl and pentaacyl species, respectively. The ion peak at *m*/*z* = 1479.6 is identified as a lipid A consisting of two GlcN, two *N*-linked C12:0 (3-OH), four O-linked fatty acids, two C10:0 (3-OH), one C12:0, and one C12:0 (2-OH). In the pentaacyl lipid A, a C10:0 (3-OH) is univocally missing ($\Delta m/z$ = 170), showing the existence of a nonstoichiometric acylation. In addition, the spectrum reveals peaks of two oxonium ions generated from the cleavage of the glucoside linkage at *m*/*z* = 712.4 and 542.2. The ion peak at *m*/*z* = 712.4 is endorsed as a triacyl fragment composed by a [GlcN-C10:3OH-C12:3OH-C12:0]$^+$ sequence, while the ion peak at *m*/*z* = 542.2 indicates the presence of two fatty acids and corresponds to a [GlcN-C12:3OH-C12:0]$^+$ sequence, lacking a C10:0 (3-OH).

Since the oxonium ions arise from GlcN II, the C10:0 (3-OH) must be missing at the C3′ position which is the partial acylation site. Consequently, the C12:0 is linked to the β-position of the amide-linked fatty acid (Figure 2).

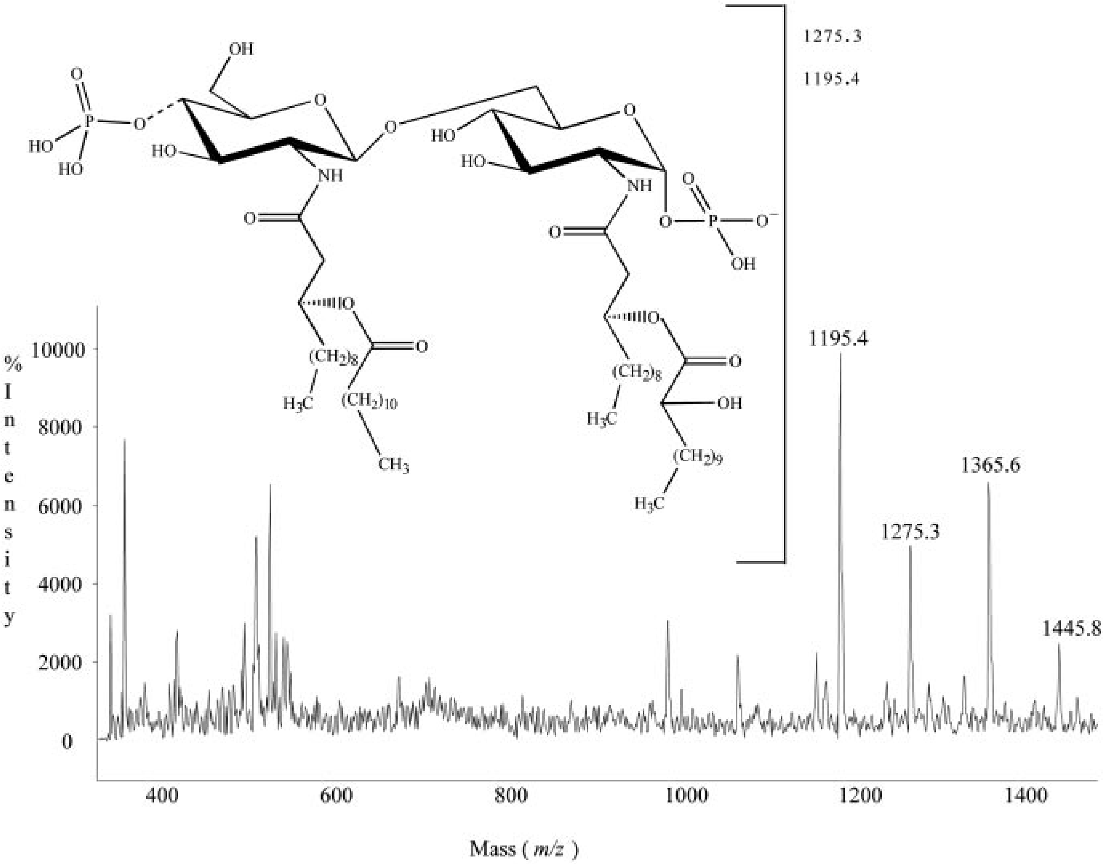

Figure 3. Negative ion MALDI-TOF mass spectrum of ammonium-treated lipid A from *P. cichorii*; the inset represents the major ion peaks at *m*/*z* = 1275.3 and 1195.4; the higher mass ion peaks at *m*/*z* = 1365.6 and 1445.8 derive from incomplete de-*O*-acylation; the dotted line indicates the nonstoichiometric phosphorylation at C-4′

The location of the fatty acids on GlcN I was univocally assigned by a mild hydrolysis with 10.7% ammonium hydroxide at room temperature overnight. This procedure allows the selective splitting of the acyl- and acyloxyacyl esters, leaving the acyl- and acyloxyacyl amides unaffected (a paper showing the general applicability of this method has been submitted[19]). The MALDI-TOF negative ion spectrum of the product shows two ion peaks at *m*/*z* = 1275.3 and 1195.4 (Δ*m*/*z* = 80), bearing two and one phosphate groups, respectively. These peaks are indicative of tetraacyl species carrying two acyloxyacyl amides, in which the secondary fatty acids are C12:0 (2-OH) and C12:0, both linked to the β-position of the amide-linked C12:3OH (Figure 3).

The positive MALDI spectrum (not shown) reveals the presence of two oxonium ion peaks at *m*/*z* = 622.1 and 542.7 (Δ*m*/*z* = 80). The peak at *m*/*z* = 622.1 is attributed to a [P-GlcN-C12:3OH-C12:0]$^+$ sequence. The corresponding oxonium ion peak at *m*/*z* = 542.7 is lacking the mass of the phosphate group (Δ*m*/*z* = 80) and is symptomatic of a partial phosphorylation at the C4′ position.

The MALDI spectrum of the intact lipid A (Figure 4) is in full agreement with the structural hypothesis, showing four ion peaks at *m*/*z* = 1616.2, 1536.2, 1446.0, 1366.0. The ion peaks at *m*/*z* = 1616.2 and 1536.2 are endorsed as the bis- and mono(phosphorylated) hexaacyl species respectively, while the ion peaks at *m*/*z* = 1446.0 and 1366.0 are identified as the bis- and mono(phosphorylated) pentaacyl species, respectively. According to the intensity of the peaks in the MALDI spectrum, the pentaacyl species were present in higher amounts.

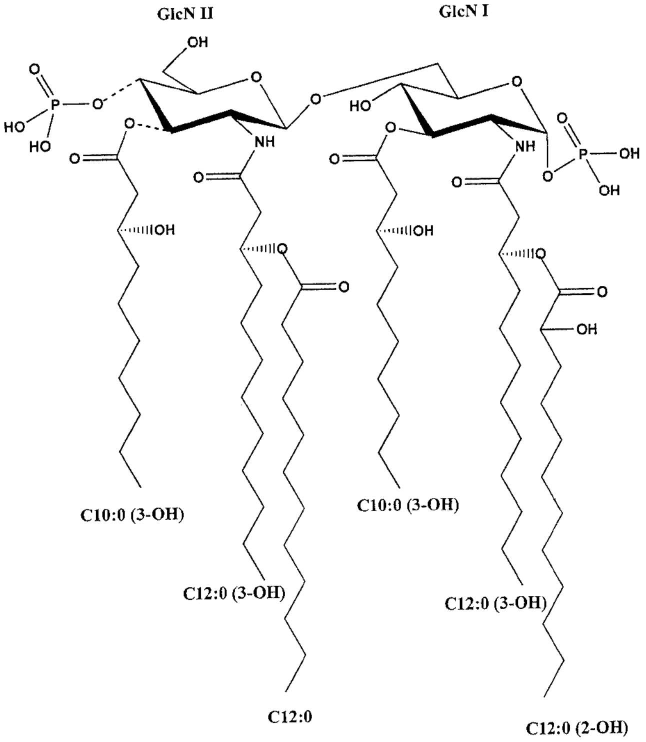

Figure 5. The main species of lipid A from *Pseudomonas cichorii* explaining the ion peaks in the negative MALDI-TOF mass spectrum in Figure 4; the dotted lines represent nonstoichiometric substitution

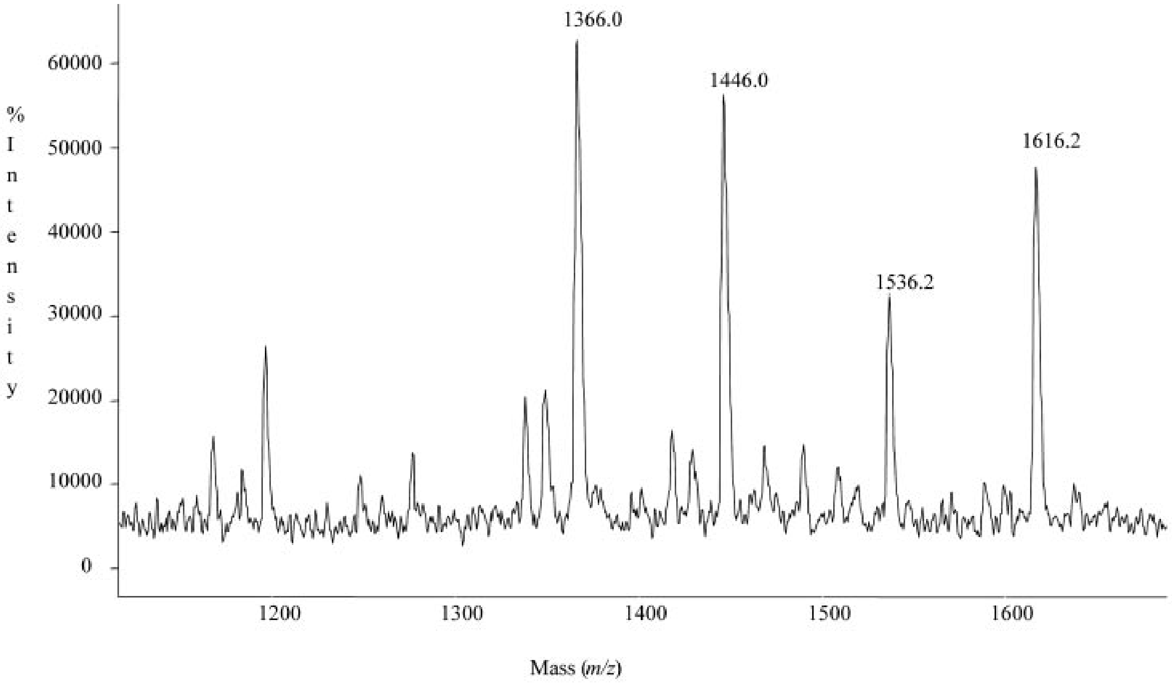

Figure 4. Negative-ion MALDI-TOF mass spectrum of intact lipid A fraction from *P. cichorii*; the ion peaks at *m*/*z* = 1616.2 and 1536.2 refer to the bis- and mono(phosphorylated) hexaacyl species, while the ion peaks at *m*/*z* = 1446.0 and 1366.0 refer to the bis- and mono(phosphorylated) pentaacyl species

Thus, these data definitively show that the intact lipid A of *Pseudomonas cichorii* is consisting of a β-(1→6)-D-glucosamine disaccharide, is acylated by C12:0 (3-OH) at the C2 and C2′ positions and by C10:0 (3-OH) at the C3 and C3′ positions. This last substitution is nonstoichiometric; C12:0 (2-OH) and C12:0 are linked to both C12:0 (3-OH) on GlcN II and GlcN I, respectively. The phosphate groups are bound at the C1 and partially at the C4′ positions (Figure 5).

Conclusions

The search for other lipid A structures of nontoxic Gram-negative bacteria is particularly important in order to obtain lipid A molecules which can act as antagonists of cell response, preventing the septic shock in mammalian cells.

The fatty acid composition of lipid A from *Pseudomonas cichorii* is very close to that of other related *Pseudomonas* species in which the main molecular species harbour five or six fatty acids.[1] The main peculiarity is that in this lipid A the acyl moiety at the C3′ position of GlcN II is partly missing.

Several studies have confirmed the importance of the structure and composition of acyl chains for biological activity and stimulation of mammalian cells; for example *P. aeruginosa* lipid A exhibits a low endotoxic activity mainly because its characteristic fatty acid composition lacks the 3-*O*-linked fatty acid at GlcN I.[12] It will be very interesting to check the biological activity of *P. cichorii* and work is now in progress to investigate this.

In *P. aeruginosa*, *Rhizobium leguminosarum*, and *Salmonella typhimurium*, a lipase has been found in the external membrane that cleaves the C-3 ester linkage after complete biosynthesis of the lipid A.[13,14] In analogy, a different lipase should be present in the outer membrane of *P. cichorii*, able to cleave selectively the ester-bound fatty acid of the distal GlcN. The discovery of this new unidentified enzyme could provide a new biochemical apparatus for selective de-*O*-acylation and preparation of new lipid A derivatives which can reduce immune stimulation in animal systems.

From a phytopathological point of view, this chemical peculiarity in bacteria could play an important role for the bacterium in the infected host. Indeed, plants have been found to have systems of innate immunity,[15,16] and it is intriguing that in *R. leguminosarum* the absence of the 3-*O*-acyl fatty acid helps the bacterium to evade the host's response while the plant can still defend itself against other Gram-negative infections.[13] Analogously, the absence of a 3′-*O*-acyl fatty acid in the unusual lipid A of *P. cichorii* might be a strategy by which the bacterium eludes the immune response. Further studies are needed to confirm this hypothesis.

Despite several articles in the literature on lipid A structural determinations, the combined use of MALDI-MS and NMR to obtain the fine structure of this key glycolipid has not often been performed. Moreover, the mild ammonium hydrolysis method used in combination with MALDI-MS is a new useful tool to afford the secondary fatty acids distribution, a problem which has been very difficult to solve so far.

Experimental Section

Bacterial Strains, Growth Conditions and Isolation of LPS: *P. cichorii* strain 5707 (from *Cichorium endiva* L.) was grown in IMM;[17] 8 mL of bacterial suspension (A_{600} = 0.35) was added to 1-L Erlenmeyer flasks containing 400 mL of medium and incubated at 25 °C. The bacterial suspension was centrifuged (9000 *g* × 20 min) and the harvested cells were washed three times with 0.85% NaCl and lyophilised. The lipopolysaccharide was recovered in the phenol phase[6] according to the hot phenol/water method as described.[5]

Preparation of Free Lipid A: Free lipid A was obtained by treatment of LPS with 0.1 M sodium acetate buffer (pH = 4.4) containing 1% SDS for 2 h at 100 °C. The solution was lyophilised, treated with HCl/ethanol to remove the detergent, dried, re-dissolved in water and centrifuged (4 °C, 90 min, 100000 *g*). The obtained precipitate was lipid A.

Compositional Analysis: Phosphate analysis was carried out according to the published method.[7] The monosaccharides were identified as acetylated *O*-methyl glycosides derivatives. The sample was methanolysed with HCl/MeOH (1 M) at 80° for 24 h, then dried and extracted with MeOH/hexane. The methanolic phase, containing the *O*-methyl glycosides, was acetylated and analysed by GC-MS. The absolute configuration of the monosaccharides was determined on acetylated glycosides of (+)-2-octanol obtained according to the published method.[7] Methylation analysis was carried out on dephosphorylated and reduced product; the sample (1 mg) was kept at 4 °C, 48 h, in HF 48% (200 μL) and then concentrated under a stream of nitrogen. The residue was dissolved in water and 1 drop of pyridine and reduced with $NaBH_4$ for 18 h. After workup, methylation was performed with methyl iodide as published.[18] The hydrolysis of the methylated sugar backbone was performed with 4 M TFA (100 °C, 4 h) and the partially methylated product, after reduction with $NaBH_4$, was converted into alditol acetates with acetic anhydride in pyridine at 80 °C for 30 min and analysed by GC-MS as described later.

The total fatty acid content was obtained by acid hydrolysis; lipid A was treated first with HCl (4 M), 4 h, 100 °C, then with NaOH (5 M), 30 min, 100 °C; the pH was adjusted to acidity and the fatty acids were extracted with $CHCl_3$, methylated with diazomethane and analysed by GC-MS. The ester-bound fatty acids were selectively cleaved by hydrolysis with NaOH (0.5 M)/MeOH (1:1, v/v), 85 °C, 2 h; the product was acidified, extracted with $CHCl_3$, methylated with CH_2N_2 and analysed by GC-MS.[8] The absolute configuration of fatty acids was determined as described.[10,11]

De-*O*-acylation and Dephosphorylation of Lipid A: Lipid A was de-*O*-acylated in THF with anhydrous hydrazine at 37° for 1.5 h, then the suspension was treated with cooled acetone and centrifuged at 5000 *g*, 15 min; the precipitate was the de-*O*-acylated product. Alternatively, a mild de-*O*-acylation was carried out by incubation of the product (200−500 μg) with 10.7% ammonium hydroxide (200 μL) at 20 °C for 16 h. The dephosphorylation of lipid A was performed with HF (48%). Briefly, an aliquot of the product was stirred at 4 °C for 48 h in HF (48%), then the product was dried under vacuum.

MALDI-TOF Analysis: MALDI-TOF analyses were conducted using a Perseptive (Framingham, MA, USA) Voyager STR instrument equipped with delayed extraction technology and with a reflectron. Ions formed by a pulsed UV laser beam (nitrogen laser, $\lambda = 337$ nm) were accelerated by 20 kV. The mass spectra reported are the result of 128 laser shots, and mass accuracy was less than 10 ppm in reflectron mode. Insulin and myoglobin were used for external calibration. The dried samples were dissolved in $CHCl_3$/CH_3OH (50:50, v/v) at a concentration of 25 pmol·mL^{-1}. The matrix solution was prepared by dissolving 2,5-dihydroxybenzoic acid (DHB) in CH_3OH at a concentration of 30 mg·mL^{-1} or trihydroxyacetophenone (THAP) in CH_3OH/0.1% trifluoroacetic acid/CH_3CN (7:2:1) at a concentration of 75 mg·mL^{-1}. A sample/matrix solution mixture (1:10, v/v) was deposited (1 mL) onto a stainless steel gold-plated 100-sample MALDI probe tip, and dried at room temperature.

NMR Spectroscopy: The ^{1}H, ^{13}C, and ^{31}P NMR spectra of de-*O*-acylated lipid A were obtained at 343 K in [D$_6$]DMSO at 400, 100, and 162 MHz, respectively, with a Bruker DRX 400 spectrometer equipped with a reverse probe. ^{13}C and ^{1}H NMR chemical shifts are expressed in δ relative to dimethyl sulfoxide ($\delta_H = 2.49$ ppm, $\delta_C = 39.7$ ppm), while ^{31}P NMR chemical shifts are relative to external standard (phosphoric acid). Two-dimensional spectra (COSY, TOCSY, ROESY, HSQC) were measured using standard Bruker software. All homonuclear experiments were performed acquiring 4096 data points in the *F*2 dimension with 512 experiments in *F*1. The data matrix was zero-filled in the *F*1 dimension to give a matrix of 4096 × 2048 points and was resolution-enhanced in both dimensions by a shifted sine-bell function before Fourier transformation. The TOCSY experiment was performed with a mixing time of 80 ms, while a mixing time of 300 ms was used in the ROESY experiment. The heteronuclear experiment was performed using pulse field gradient program as gHSQC.

Gas Chromatography: GC was performed with a Hewlett−Packard 5890 instrument, SPB-5 capillary column (0.25 mm × 30 m, Supelco), for methylation analysis of sugars the temperature profile was 150 °C for 5 min, then 5 °C·min^{-1} to 300 °C, while for monosaccharide absolute configuration analysis it was 150 °C for 8 min, then 2 °C·min^{-1} to 200 °C for 0 min, then 6 °C·min^{-1} to 260 °C for 5 min. For fatty acids analyses the temperature profile was 150 °C for 3 min, then 10 °C·min^{-1} to 280 °C over 20 min.

Acknowledgments

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[1] U. Zähringer, B. Lindner, E. T. Rietschel, in *Endotoxin in Health and Disease, Chemical Structure of Lipid A: Recent Advances in Structural Analysis of Biologically Active Molecules* (Eds: D. C. Morrison, H. Brade, S. Opal, S. Vogel), M. Dekker, Inc., New York, **1999**, chapter 7.

[2] M. A. Newmann, E. Von Roepenack, M. Daniels, M. Dow, *Mol. Plant Pathol.* **2000**, *1*, 25−31.

[3] W. J. Christ, O. Asano, A. L. Robidoux, M. Perez, Y. Wang, G. R. Dubuc, W. E. Gavin, L. D. Hawkins, P. D. McGuinness, M. A. Mullarkey, M. D. Lewis, Y. Kishi, T. Kawata, J. R. Bristol, J. R. Rose, D. P. Rossignol, S. Kobayashi, I. Hishinuma, A. Kimura, N. Asakawa, K. Katayama, I. Yamatsu, *Science* **1995**, *268*, 80−83.

[4] J. F. Bradbury, in *Guide to Plant Pathogenic Bacteria*, CAB International Mycological Institute, Kew, England, **1986**.

[5] O. Westphal, K. Jann, *Methods Carbohydr. Chem.* **1965**, *5*, 83−91.

[6] J.J. Barbero, C. de Castro, A. Evidente, A. Molinaro, M. Parrilli, G. Surico, *Eur. J. Org. Chem.*, in press.

[7] K. Leontein, J. Lönngren, *Methods Carbohydr. Chem.* **1978**, *62*, 359−362.

[8] W. Kaca, J. de Jongh-Leuvenink, U. Zähringer, E. T. Rietschel, H. Brade, J. Verhoef, V. Sinnwell, *Carbohydr. Res.* **1988**, *179*, 289−299.

[9] H. W. Wollenweber, E. T. Rietschel, *J. Microbiol. Methods* **1990**, *11*, 195−211.

[10] E. T. Rietschel, *Eur. J. Biochem.* **1976**, *64*, 423−428.

[11] K. Bryn, E. T. Rietschel, *Eur. J. Biochem.* **1978**, *86*, 311−315.

[12] V. A. Kulshin, U. Zähringer, B. Lindner, K. E. Jager, B. Dmitriev, E. T. Rietschel, *Eur. J. Biochem.* **1991**, *198*, 697−704.

[13] S. S. Basu, K. A. White, N. L. Que, C. R. Raetz, *J. Biol. Chem.* **1999**, *274*, 11150−11158.

[14] M. S. Trent, W. Pabich, C. R. Raetz, S. I. Miller, *J. Biol. Chem.* **2001**, *276*, 9083−9092.

[15] M. A. Newmann, M. Daniels, M. Dow, *Mol. Plant Microbe Interact.* **1997**, *10*, 812−820.

[16] M. A. Newmann, M. Daniels, M. Dow, *Mol. Plant Microbe Interact.* **1995**, *8*, 778−780.

[17] G. Surico, P. Lavermicocca, N. S. Iacobellis, *Phytopathol. Mediterr.* **1988**, *27*, 163−168.

[18] I. Ciucanu, F. Kerek, *Carbohydr. Res.* **1984**, *131*, 209−217.

[19] A. Silipo, R. Lanzetta, A. Amoresano, M. Parrilli, A. Molinaro, *J. Lip. Res.*, in press.

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