JOURNAL OF BACTERIOLOGY, Dec. 1990, p. 6631–6636
0021-9193/90/126631-06$02.00/0

Vol. 172, No. 12

Structural Studies of Lipid A from *Pseudomonas aeruginosa* PAO1: Occurrence of 4-Amino-4-Deoxyarabinose

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Received 9 May 1990/Accepted 5 September 1990

Lipid A derived from *Pseudomonas aeruginosa* PAO1 contains a biphosphorylated 1-6-linked glucosamine disaccharide backbone. The reducing glucosamine has an unsubstituted glycosidically linked phosphate at C-1. The nonreducing glucosamine has an ester-bound phosphate at C-4′ which is nonstoichiometrically substituted with 4-amino-4-deoxyarabinose. Induction of 4-amino-4-deoxyarabinose was dependent on cultural conditions. No pyrophosphate groups were detected. Acyloxyacyl diesters are formed by esterification of the amide-bound 3-hydroxydodecanoic acid with dodecanoic acid and 2-hydroxydodecanoic acids in an approximate molar ratio of 2:1. Dodecanoic and 3-hydroxydecanoic acids are esterified to positions C-3 and C-3′ in the sugar backbone. All hydroxyl groups of the glucosamine disaccharide except C-4 and C-6′ are substituted. Lipopolysaccharide chemical analyses measured glucose, rhamnose, heptose, galactosamine, alanine, phosphate, and glucosamine. The proposed lipid A structure differs from previous models. There are significant differences in acyloxyacyl diesters, and the proposed model includes an aminopentose substituent.

Lipopolysaccharides (LPS) are major components of the gram-negative bacterial outer membrane and are essential for the assembly, organization, and functioning of this vital structure. LPS contributes to the pathophysiology of the notorious opportunistic pathogen *Pseudomonas aeruginosa* by functioning as a virulence factor and acting in consort with other outer membrane constituents to form a permeability barrier against antimicrobial agents (14).

P. aeruginosa LPS is compositionally similar to enterobacterial LPS (12). These structural similarities include a biphosphorylated D-glucosamine disaccharide lipid A backbone, an oligosaccharide core, and serologically diverse O chains. However, *P. aeruginosa* LPS differs from enterobacterial LPS by having a large number of phosphate residues, by the presence of L-alanine in the core, and by the occurrence of unusual sugars and amino compounds in the O chain (15, 20).

Enterobacterial lipid A's have a number of features that serve to distinguish them from their pseudomonal counterparts. One such structure is 4-amino-4-deoxyarabinose (4-AraN), which is found in some but not all enteric lipid A's. The physiological function of 4-AraN is unresolved, although it has been suggested by Vaara et al. to play a role in resistance against selected antibiotics by substituting onto lipid A phosphate groups (28, 29). In this capacity the cationic 4-AraN could facilitate resistance by either repulsing positively charged antimicrobial agents such as polymyxin B or by occupying their LPS attachment sites. Vaara et al. also proposed that resistance to polymyxin B is proportional to the number of lipid A phosphate groups substituted with 4-AraN. 4-AraN substitution in LPS has been previously reported in selected members of the family *Enterobacteriaceae*, in *Chromobacterium violaceum*, and in phototrophic bacteria (8, 22, 26, 27).

In this study we report the isolation and partial characterization of LPS and lipid A from *P. aeruginosa* PAO1. We present evidence establishing the presence of 4-AraN and demonstrate that 4-AraN substitutes onto lipid A phosphate groups. Based on these data we propose a structural model of *P. aeruginosa* lipid A.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *P. aeruginosa* PAO1 and polymyxin B-resistant derivatives (isolate A, isolate A reverted) have been previously described (7). *P. aeruginosa* isolates obtained from patients with cystic fibrosis have been described by Fomsgaard et al. (5).

P. aeruginosa PAO1 and strains derived from PAO1 were cultured in two different minimal salts media. PAO1, isolate A, and isolate A reverted were grown in the glucose-salts basal medium (BM) of Gilleland and Lyle (7) as described by Conrad and Galanos (4). Strain PAO1 was also cultivated in 6- and 14-liter batches in a microferm apparatus (New Brunswick Scientific Co., Edison, N.J.) operated at 37°C with aeration. The fermentor growth medium was DO-minimal medium, which contained (per liter) 3.5 g of K_2HPO_4, 1.0 g of KH_2PO_4, 0.5 g of sodium citrate, 1.0 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4$, and 4.0 g of D-glucose (pH 7.2). Cells were harvested in the middle to late log phase (ca. 7 h), washed once with water, and freeze-dried. Batches of strain PAO1 were also grown at 37°C on complex solid medium (1% meat extract, 4% agar). At 48 h, cultures were harvested from petri plates with water, washed once, and freeze-dried. The cystic fibrosis isolates of *P. aeruginosa* were cultured on Trypticase soy agar and harvested (5).

LPS extraction. The *P. aeruginosa* strains used in this study are heterogenous with respect to smooth and rough LPS forms (4, 5) and responded differentially to the various techniques used to extract LPS. The preferred method of LPS extraction was determined for each strain by the sequential application of the phenol-chloroform-petroleum ether method of Galanos et al. (6), the phenol-water procedure of Westphal and Jann (32), and the modified phenol-

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water procedure of Conrad and Galanos (4). Cells that were not successfully extracted by the initial protocol (phenol-chloroform-petroleum ether) were washed with acetone and then extracted with phenol-water. If necessary, this residue was washed with acetone and extracted by the modified phenol-water procedure.

LPS was extracted from cells of strain isolate A by the phenol-chloroform-petroleum ether method. LPS was extracted from isolate A reverted and from the cystic fibrosis isolates by the phenol-water procedure. LPS was extracted from strain PAO1 by the modified phenol-water technique. Structural and biochemical analyses were performed with LPS extracted from PAO1 cells grown in 2-liter batches of BM. LPS from *Proteus mirabilis* and *Rhodospirillum tenue* were kindly provided by H. Mayer (Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany).

Isolation and purification of lipid A. Lipid A was released from LPS by 1% acetic acid (100 mg/20 ml, 100°C, 2 h). The sediment was cooled in an ice bath, centrifuged in a table top centrifuge (8,000 rpm, 4°C, 15 min), and washed successively with warm water (50°C) and acetone. Crude lipid A was purified by dissolution in chloroform, filtration, concentration to near dryness by a gentle stream of nitrogen, and precipitation with 3 volumes of acetone and then freeze-dried.

Analytical methods. Fatty acids from intact LPS were analyzed by gas-liquid chromatography (GLC) by the methods of Wollenweber et al. (34) and Rietschel et al. (17, 18). Complete conversion to the methyl ester was ensured in all preparations by the addition of the diazomethane reagent (16). Heptadecanoic and 2-hydroxytetradecanoic acids were used as internal standards. The content of 3-deoxy-D-manno-2-octulosonic acid (KDO) was determined by the method of Karkhanis et al. (9) with the ammonium salt of KDO (Sigma Chemical Co., St. Louis, Mo.) as a standard. Neutral sugars of LPS were analyzed by GLC by the protocol of Strittmatter et al. (24). The alditol acetate derivatives were prepared for GLC by the method of Sawardeker et al. (21). Xylose was added as an internal standard. Total phosphate was determined by the method of Lowry and Tinsley (13). Alanine, amino sugars, and their phosphate derivatives were liberated from LPS and lipid A by acid hydrolysis (4 M HCl, 100°C, 18 h) and characterized by a Kontron chromacon 500-amino-acid analyzer with an Anacomp 220 computer. Glucosamine was analyzed by the method of Strominger et al. (25) and with an automatic amino acid analyzer as described above. Glucosamine phosphate derivatives in lipid A were characterized by two experimental conditions. In the first protocol lipid A was subjected to acid hydrolysis as described above for amino compounds. In the second protocol lipid A underwent mild alkaline treatment (0.2 M NaOH, 100°C, 1 h) before acid hydrolysis as described above.

Structural analysis of lipid A was performed by permethylation of free lipid A with dimsyl potassium-methyl iodide by the Hakomori procedure as described by Waeghe et al. (31). Unsubstituted free hydroxyl groups in the lipid A sugar backbone were localized by silica gel-catalyzed diazomethane methylation of free lipid A by the method of Tharanathan et al. (26). Methylation was followed by acid hydrolysis (4 M HCl, 100°C, 8 h), N-acetylation, reduction, and O-acetylation. All preparations were analyzed by combined GLC and mass spectrometry (MS).

GLC-MS was performed on a Finnigan quadrupole MAT 1020 model B instrument by electron ionization fitted with an automatic data system. Amino sugar derivatives were analyzed on a CP-SIL-5 column (25 m long, 0.25-mm inner diameter) with a temperature program from 85 to 250°C (5°C/min). Fatty acid methyl esters were separated on a WCOT SE-54 column (25 m long, 0.25-mm inner diameter) with a temperature program from 140 to 280°C (10°C/min). The carrier gas for both columns was helium, the injector temperature was 250°C, transfer-line temperature 280°C, and the electron energy was 70 eV. ^{31}P nuclear magnetic resonance spectra were recorded on a Bruker WM 300 spectrometer in the Fourier-transform mode at 121.51 MHz. Chemical shifts were measured relative to external 85% phosphoric acid (0.00 ppm). Samples (5 mg) were dissolved in ^2H_2O (2.5 ml) containing 5 mM EDTA and 2% deoxycholate (pH 7.3). Spectra were recorded for free lipid A, lipid A titrated from pH 7.3 to 10.0 (0.1 M NaOH), and previously alkali-treated lipid A (0.25 M NaOH, 20°C, 16 h). Analyses of the amide-bound acyloxyacyl residues were done by a Kraska methylation procedure modified as described by Wollenweber et al. (33).

Identification of 4-AraN. 4-AraN was released from lipid A and LPS by acid hydrolysis under mild conditions (0.01 M HCl, 100°C, 10 min) and by a rapid method. In the rapid method, 500 μg of freeze-dried material (LPS or lipid A) was added to 50 μl of 10 M HCl, sonicated for 5 s, and immediately immersed in a boiling water bath for 15 s. The reaction mixture was diluted with 250 μl of water, and the reaction was quenched in an ice bath. Particulate material from both procedures was immediately removed by centrifugation and discarded. After evaporation with nitrogen, acid hydrolysates (supernatants) were analyzed with an automatic amino acid analyzer as described by Volk et al. (30). Authentic 4-AraN for comparison was isolated from *P. mirabilis* and *R. tenue* LPS. Aminopentose-phosphate was released from LPS by alkaline hydrolysis (0.2 M NaOH, 100°C, 1 h). Acid and alkaline hydrolysates of lipid A were subjected to preparative high-voltage paper electrophoresis by the method of Kickhöfen and Warth (10) with pyridine-acetic acid-water (10:4:86, vol/vol/vol) (pH 5.3) as the buffer. Amino sugars were visualized with ninhydrin, ammonium molybdate, and alkaline silver nitrate (Trevelyan reagent). Spots that were positive with ninhydrin and ammonium molybdate but negative with silver nitrate were preparatively eluted with 0.01 M HCl, treated with 0.3 U of alkaline phosphatase (50 mM Tris hydrochloride [pH 8.0], 56°C, 3 h), and reelectrophoresed by the same protocol. Additional evidence for the identification of 4-AraN was obtained by GLC-MS. LPS was hydrolyzed by acid (0.02 M HCl, 100°C, 10 min), N-acetylated by the method of Kozulic et al. (11), reduced with $NaBH_4$, and then analyzed by GLC-MS.

RESULTS

Isolation and characterization of 4-AraN. An aminopentose identified as 4-AraN was released by acid hydrolysis from the LPS of *P. aeruginosa* PAO1. This identification was based on the following data. High-voltage paper electrophoresis of the hydrolysate yielded a spot with a mobility (relative to glucosamine) of 1.18 which stained purplish-black with the Trevelyan reagent and yellowish-brown with ninhydrin. Analysis of the hydrolysate with an automatic amino acid analyzer showed a single peak with an A_{440}/A_{570} ratio of 6.0. Additional evidence for the identification of 4-AraN was provided by GLC-MS. GLC-MS analysis of mildly acid-hydrolyzed LPS gave a single peak with characteristic fragments at *m/z* 144, 145, 217, and 288, as would be expected for the alditol acetate derivative of 4-AraN reduced

by $NaBH_4$ (8). These physical characteristics corresponded with authentic 4-AraN isolated from *R. tenue* LPS during parallel experiments. The two methods for acid hydrolysis (mild conditions and the rapid method) were equally effective in releasing 4-AraN from LPS. 4-AraN was not quantitated due to the lack of proper standards necessary to obtain the molar response factors in GLC analysis with the flame ionization detector.

Localization of 4-AraN. 4-AraN was found only in the lipid A moiety of LPS and was detected in neither the core nor the side chain fraction. The location of 4-AraN on the lipid A disaccharide backbone was elucidated by the following protocol. The complete acid hydrolysis of LPS yielded glucosamine and glucosamine 6-phosphate in an approximate molar ratio of 5:1 (251 and 54 nmol, respectively, per mg of LPS). The incidence of glucosamine 6-phosphate has been explained by Rietschel et al. (17) as an acid-catalyzed rearrangement during which the ester-linked phosphate on C-4′ of the nonreducing glucosamine migrates to C-6′. Glucosamine phosphate was not detected in LPS that had been subjected to mild alkaline treatment before acid hydrolysis. This absence may be explained by the alkali-mediated hydrolysis of ester-linked phosphate groups, which have a substituent that prevents the formation of a cyclic phosphate group. If this substituent were 4-AraN, hydrolysis should yield 4-amino-4-deoxyarabinose-1-phosphate. The presence of this compound was confirmed by the following series of experiments.

LPS was hydrolyzed by alkali (0.2 M NaOH, 100°C, 1 h), and the hydrolysate was subjected to preparative high-voltage paper electrophoresis. A significant spot remaining at the origin was ninhydrin and ammonium molybdate positive but silver nitrate negative. This spot was eluted with 0.01 M HCl, treated with alkaline phosphatase, and electrophoresed. The material then migrated exactly like the authentic 4-AraN control, with positive reactions to ninhydrin and silver nitrate and a negative reaction to ammonium molybdate. This was consistent with the material cleaved during alkaline hydrolysis being 4-amino-4-deoxyarabinose-1-phosphate. Therefore, 4-AraN is bound by glycosidic linkage to the ester-bound phosphate of the nonreducing glucosamine in the lipid A backbone.

Location of phosphates on lipid A. The locations of phosphate groups on the lipid A disaccharide backbone were studied by ^{31}P nuclear magnetic resonances. Results were interpreted by the criteria of Baltzer and Mattsby-Baltzer (1), Batley et al. (2), Bhat et al. (3), and Strain et al. (23). Figure 1 shows the spectra of free lipid A and alkaline-treated lipid A. Four signals were observed for free lipid A, resonating at −1.56, +2.85, +3.25, and +5.00 ppm. Titration of lipid A from pH 7.3 to 10.0 demonstrated that only the signal at −1.56 ppm was pH independent, whereas the other signals were shifted approximately 3 ppm downfield (data not shown). By the standards of Batley et al. (2), the signal at −1.56 ppm was assigned to a phosphodiester arising from substitution of ester-linked phosphate with 4-AraN. The other three signals were pH dependent, and the changes in chemical shifts observed during titration were attributed to the ionization of monophosphates. The monophosphate signal resonating at +5.0 ppm corresponded to an ester-linked phosphate, indicating nonmolar substitution of ester-linked phosphate by 4-AraN (3). The two signals resonating at +2.85 ppm and +3.25 ppm could be attributed to glycosidic phosphate at C-1, indicating an intrinsic heterogeneity in *P. aeruginosa* PAO1 lipid A. The ^{31}P nuclear magnetic resonance spectrum of lipid A after alkaline treatment gave only two monoester signals at +1.73 and +4.80 ppm, indicating a loss of a diester, such as ester-bound phosphate substituted with 4-AraN.

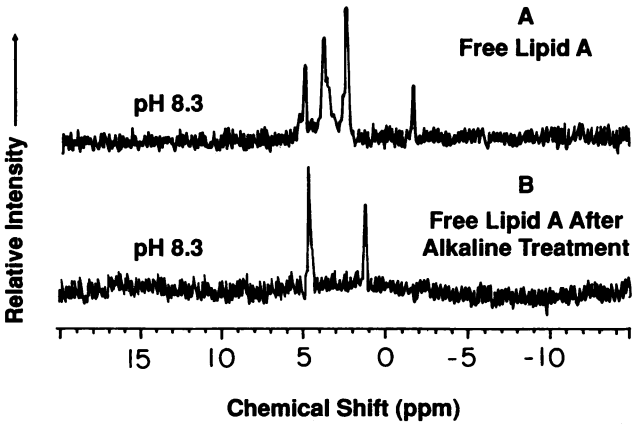

FIG. 1. ^{31}P nuclear magnetic resonance spectra of (A) free lipid A and (B) free lipid A previously subjected to mild alkali treatment (0.25 M NaOH, 100°C, 1 h). Samples (5 mg) were dissolved in 2.5 ml of ^2H_2O containing 5 mM EDTA and 2% sodium deoxycholate. Samples were titrated from pH 7.3 to 10.0 (0.1 M NaOH), and spectra were recorded at pH 8.3.

Analysis of the lipid A sugar backbone. Free lipid A was analyzed by the Hakomori procedure with permethylation, hydrolysis, and conversion into partially methylated alditol acetate derivatives. GLC-MS analysis revealed the presence of 3,4-di-*O*-methylated (reducing end) and 3,4,6-tri-*O*-methylated (nonreducing end) derivatives of 2-deoxy-2-*N*-methyl acetamido glucose. The presence of the latter derivative could be explained by the removal of an ester-linked phosphate and its 4-AraN substituent as a result of the alkaline conditions used for the methylation procedure. These results are in agreement with a 1,6-linked glucosamine disaccharide backbone for the lipid A.

Fatty acid analysis. Total fatty acids of *P. aeruginosa* PAO1 LPS were liberated by methanolysis as their methyl esters and identified as dodecanoic, 3-hydroxydecanoic, 2-hydroxydodecanoic, and 3-hydroxydodecanoic acids (Table 1). 3-Hydroxydodecanoic acid was exclusively amide bound to glucosamine, and all other fatty acids were in ester linkage as shown by sodium methoxide treatment. Trace amounts (<1%, by weight) of other fatty acids were noted. Amide-linked acyloxyacyl diesters of lipid A were released after a modified Kraska methylation and were analyzed by GLC-MS. The acyloxyacyl diesters were identified as 3-dodecanoyloxydodecanoic acid [3-*O*-(12:0)-12:0] and 3-(2-methoxydodecanoyloxy)dodecanoic acid [3-*O*-(2-OCH_3-12:0)-12:0] in an approximate molar ratio of 2:1. The former diester was characterized by fragments at *m/z* 180, 181, 183,

TABLE 1. Fatty acid composition of LPS from *P. aeruginosa* PAO1

Analytical procedure	Fatty acid composition[a] (μg/mg of LPS)			
	$C_{12:0}$	3-OH-$C_{10:0}$	2-OH-$C_{12:0}$	3-OH-$C_{12:0}$
Total	37.9 ± 3.2	54.3 ± 2.4	46.2 ± 1.6	79.6 ± 3.7
Ester bound	24.2 ± 5.4	32.3 ± 7.3	29.7 ± 6.7	0.4 ± 0.0
Amide bound	2.1 ± 0.9	2.4 ± 0.3	1.6 ± 0.1	53.1 ± 7.4

[a] Means ± standard deviations from three to six independent determinations of each analytical procedure.

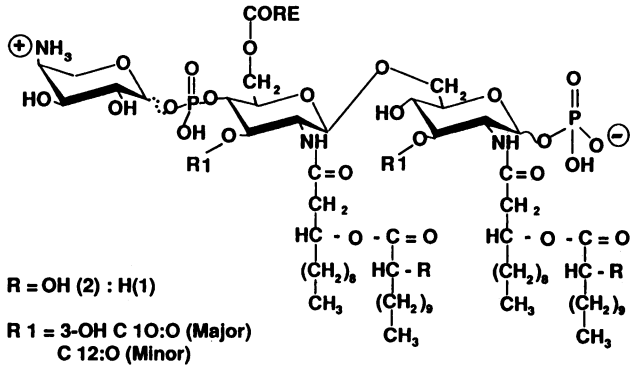

FIG. 2. Proposed structure for the lipid A of *P. aeruginosa* PAO1.

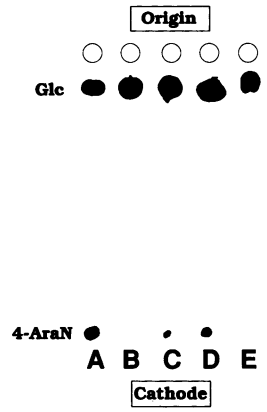

FIG. 3. Induction of 4-AraN. Acid hydrolysates of LPS extracted from a *P. mirabilis* control and *P. aeruginosa* PAO1 grown with various cultural conditions. Separation was by high-voltage paper electrophoresis, and visualization was with the Trevelyan reagent. Lanes: A, *P. mirabilis*; B, PAO1 in DO-minimal medium (14-liter batch); C, PAO1 in DO-minimal medium (6-liter batch); D, PAO1 in BM-minimal medium (2-liter batch); E, PAO1 on complex solid medium (1% meat extract, 4% agar). The origin was spotted with 80 μl of supernatant (500 μg of LPS, 300 μl of solution). The figure shows only origin and cathode portion of chromatogram, which includes glucose (Glc) and 4-AraN. 4-AraN was identified as described in Materials and Methods.

212, 213, and 229, whereas the latter diester gave fragments at *m/z* 185 and low-intensity ions at *m/z* 180, 181, 212, 213, and 229. The fragments at *m/z* 183 and 185 are indicative of substituents at the 3-hydroxyl group (34). These data indicate that amide-bound 3-hydroxydodecanoic acid is esterified with dodecanoic acid and 2-hydroxydodecanoic acid in an approximate 2:1 molar ratio. Additional evidence for this configuration was provided by characterization of LPS extracted from strains isolate A and isolate A reverted, which were derived from the *P. aeruginosa* PAO1 parent strain. These polymyxin B-resistant strains are deficient in 2-hydroxydodecanoic acid and yielded only one species of diester, which was characterized as 3-*O*-(12:0)-12:0.

Localization of free hydroxyl groups. Silica gel-catalyzed methylation of free lipid A, followed by acid hydrolysis, reduction, and acetylation, resulted in two primary derivatives identified by GLC-MS as 6-*O*-methyl-2-*N*-acetamido-2-deoxyglucitol peracetate and 4-*O*-methyl-2-*N*-acetamido-2-deoxyglucitol peracetate. By analogy to *Salmonella* lipid A (19), the C-6-methylated glucosaminitol derivative is derived from the nonreducing glucosamine with the hydroxyl group at C-6′, providing the attachment site for KDO residue(s) linking lipid A to the core oligosaccharide. Considering enteric lipid A as a model dictates that C-4-methylated glucosaminitol results from a free C-4 hydroxyl group on the reducing glucosamine residue. Undermethylation resulted in trace amounts of fully acetylated glucosaminitol acetate. The absence of di-*O*-methylated glucosaminitol derivatives indicated that, with the exception of C-4 and C-6′ as discussed above, all available positions on free lipid A are fully substituted by either fatty acid or phosphate groups. Based on these investigations, we propose the following model for *P. aeruginosa* PAO1 lipid A (Fig. 2).

Induction of 4-AraN. 4-AraN synthesis in *P. aeruginosa* PAO1 and derivatives is apparently an inductive process related to cultural conditions (Fig. 3). 4-AraN was detected in PAO1 cells grown in small batches (2 or 6 liters) of either of two different minimal media (BM and DO-minimal medium). 4-AraN was also found (data not shown) in the LPS of strains isolate A and isolate A reverted that had been cultured in 2-liter batches of BM. 4-AraN was not observed in the LPS of PAO1 cells grown in large batches (14 liters) of minimal medium or on complex solid medium. 4-AraN was not detected in the LPSs of five previously characterized *P. aeruginosa* cystic fibrosis isolates grown on complex liquid medium (5).

Chemical composition of LPS. Chemical analyses of LPS indicated that the primary neutral sugars and their respective molar ratios were glucose (3.9), rhamnose (3.5), and heptose (1.0). The principal amino compounds were glucosamine, glucosamine phosphate, galactosamine, and alanine. Phosphate was found in complete LPS, core, and lipid A. The optimal conditions for release of KDO were 1 M HCl at 100°C for 30 min. The concentrations of these components were consistent with previous analyses of *P. aeruginosa* LPS (12, 20).

DISCUSSION

On the basis of these data and by analogy to the enteric model, we propose the structural model of *P. aeruginosa* PAO1 lipid A shown in Fig. 2. Chemical characterization of lipid A components including total fatty acids, amino compounds, and phosphates were quantitatively and qualitatively similar with previous models. However, major differences were noted in the composition of the acyloxyacyl diesters, location of free hydroxyl groups, and phosphate substituents.

Previous models of *P. aeruginosa* lipid A proposed that acyloxyacyl diesters are formed from the esterification of 3-hydroxydecanoic acid by 2-hydroxydodecanoic acid on C-4 and C-6′ (12). Our methylation studies of *P. aeruginosa* free lipid A indicate that C-4 has a free hydroxyl group and that C-6′ is most likely linked to the core via KDO. Our data demonstrate that amide-bound 3-hydroxydodecanoic acid is esterified with dodecanoic acid and 2-hydroxydodecanoic acid in an approximate ratio of 2:1. C-3 and C-3′ of the glucosamine lipid A backbone are esterified with dodecanoic and 3-hydroxydecanoic acid. The lipid A backbone consists of reducing and nonreducing glucosamines bound in a 1-6 linkage.

Phosphate analyses indicated that *P. aeruginosa* PAO1 lipid A has an unsubstituted glycosidically linked phosphate at C-1 of the reducing glucosamine and an ester-bound phosphate at C-4′ of the nonreducing glucosamine. The

latter phosphate is substituted nonstoichiometrically with 4-AraN, resulting in an intrinsic heterogeneity. No pyrophosphate groups were detected by ^{31}P nuclear magnetic resonance.

This is the initial report of 4-AraN in the LPS of *P. aeruginosa*. The metabolic role of 4-AraN in gram-negative bacteria is ill defined but is thought to contribute to the overall resistance of selected enteric bacteria by occupying potential antimicrobial agent attachment sites on the acidic lipid A moiety. The inductive process of 4-AraN in *P. aeruginosa* PAO1 is unclear but is related to cultural conditions. A definitive correlation between structure and resistance was not established in this study, since 4-AraN was detected (but not quantitated) in both polymyxin-resistant strains (isolate A, isolate A reverted) and the polymyxin-susceptible PAO1 parent strain. The induction and physiological role of 4-AraN in *P. aeruginosa* will be addressed by a comparative study of strain PAO1 and genetically distinct strains in which metabolic variables such as pH, growth phase, aeration, media, batch, and innoculum size are rigidly controlled. Analyses of 4-AraN are handicapped by the lability of this compound to most extraction and quantitation procedures.

In summary we have extracted and partially characterized LPS and lipid A from *P. aeruginosa* PAO1. Based on these data, we propose a lipid A model depicting the location of phosphate groups, acyloxyacyl diesters, and 4-AraN.

ACKNOWLEDGMENTS

This work was supported in part by Senior International Fellowship grant 1 F06 TWO1121-01 BI-5 from the Fogarty International Center to R.S.C. while on sabbatical leave.

We thank Otto Lüderitz and Hubert Mayer for useful discussions.

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