Lipopoly saccharides Possessing Two 1-Glycero-d-manno-heptopyranosyl-a-(1→5)-3-deoxy-d-manno-oct-2-ulopyranosonic Acid Moieties in the Core Region

THE STRUCTURE OF THE CORE REGION OF THE LIPOPOLYSACCHARIDES FROM BURKHOLDERIA CARYOPHYLLII*

Received for publication, October 25, 2001, and in revised form, January 14, 2002
Published, JBC Papers in Press, January 14, 2002, DOI 10.1074/jbc.M110283200

Antonio Molinaro‡, Cristina De Castro‡, Rosa Lanzetta‡, Antonio Evidente¶, Michelangelo Parrilli||, and Otto Holst§

From the ‡Dipartimento di Chimica Organica e Biochimica, Università degli studi di Napoli “Federico II,” I-80126 Napoli, Italy, the ¶Division of Structural Biochemistry, Research Center Borstel, Center for Medicine and Biosciences, D-23845 Borstel, Germany, and the ||Dipartimento di Scienze Chimico-Agrarie, Università degli studi di Napoli “Federico II,” I-80055 Portici, Italy

Burkholderia caryophyllii is a phytopathogenic Gram-negative bacterium that had earlier been included in the genus Pseudomonas (1). However, application of ribosomal RNA (rRNA) similarity studies showed that this original genus Pseudomonas is diverse and contains five distantly related groups. Of these, RNA group I contains the members of the true genus Pseudomonas. The new genus Burkholderia (RNA group II) contains species that are either plant or animal pathogens. B. caryophyllii is responsible for the wilting of carnation (2), and it shares with other Gram-negative species the presence of lipopolysaccharides (LPSs)1 in its cell wall. One characteristic feature of LPSs from the genus Burkholderia is the occurrence of two different O-specific polysaccharides. In the case of LPSs from B. caryophyllii, two linear homo-polysaccharides were identified as O-specific polysaccharides, one of which is furnished from 3,6,10-trideoxy-4-C-(d-glyceral-1-hydroxyethyl)-d-erythro-d-gulo-decose (caryophyllose, a-1→7-linked, caryophyllan) and the other from 4,8-cyclo-3,9-dideoxy-l-erythro-d-idino-nonose (caryose, a-1→7-linked, caryan) (3–6). The caryan is acetylated in nonstoichiometric amounts, leading to a block pattern and, thus, to the establishment of repeating units in a homopolymer (7), while only the side chain the caryophyllan is randomly acetylated, and no chemical repeating unit was possible to define (8).

In LPSs, the O-specific polysaccharide is linked to the core region, which in turn is bound to the lipid A (9). All core regions identified so far (10) possess at least one residue of 3-deoxy-d-manno-oct-2-ulopyranosonic acid (Kdo) that links this region to the lipid A (Kdo I). A second characteristic molecule of the core region is 1-glycero-d-manno-heptose (Hep); however, there are heptose-free LPSs (e.g. of the genera Chlamydia and Acinetobacter). In those cases Hep is present, Kdo I is usually substituted by a Hep residue at O-5, regardless to the number of Kdo residues in the structure, and elongation of the core occurs from this Hep residue. Thus, the presence of one Hep-(α-(1→5))-Kdo moiety is a characteristic feature of heptose-containing core regions of LPSs. Kdo I may further be substituted at O-4 by a second Kdo residue (Kdo II, e.g. in Salmonella enterica and

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This article is dedicated to Professor Lorenzo Mangoni on the occasion of his 70th birthday.

‡To whom correspondence should be addressed: Analytical Biochemistry, Research Center Borstel, Parkallee 22, D-23845 Borstel, Germany. Tel.: 49-4537-188472; Fax: 49-4537-188419; E-mail: oholst@fz-borstel.de.

1 The abbreviations used are: LPS, lipopolysaccharide; Kdo, 3-deoxy-d-manno-oct-2-ulopyranosonic acid; Hep, 1-glycero-d-manno-heptose; HPAEC, high-performance anion-exchange chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry; HMQCl, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond correlation; MALDI-TOF, matrix-assisted laser desorption ionization-time-of-flight.
Escherichia coli). In a few cases, Kdo II is further substituted by neutral sugar residues, e.g., l-rhamnose or d-galactose, in particular E. coli strains. As elucidated best for LPSs of E. coli (11–13), biosynthesis of the initial parts of the core region begins with the attachment of the Kdo-α-(2→4)-Kdo disaccharide to tetraacetyl-lipid A (precursor IV8). After completion of the acylation of lipid A, the first Hep is attached to O-5 of Kdo I, followed by further elongation steps.

LPSs of plant-pathogenic bacteria have been shown to play a role in phytopathogenicity (14). Since there is only little information on the structure-function relationship of LPSs from B. caryophylli, we have begun with the characterization of its LPSs. Here, we report the structure of the core region which possesses as a novel feature two Hep-α-(1→8)-Kdo moieties.

EXPERIMENTAL PROCEDURES

Bacteria and Bacterial LPSs—B. caryophylli strain NCPP 2151 was cultivated as described previously (3). The LPSs were obtained from lyophilized bacteria by the phenol/water extraction method as described previously (3) (yield: 6% of the bacterial dry mass).

Isolation and Characterization of Oligosaccharides—The LPSs (280 mg) were hydrolyzed in 1% acetic acid (100 °C, 2 h) and the precipitate (lipid A) was removed by centrifugation (8000 × g, 30 min). The supernatant was separated by gel-permeation chromatography on a column (50 × 3 cm) of Sephadex G-50 (Amersham Biosciences, Inc.). Three fractions were obtained, the first of which eluted in the void volume and contained the caryan-attached to the core region. A second fraction consisted of a mixture of caryophyllose oligosaccharides of molecular masses higher than trisaccharides. The third fraction (27.1 mg, 34% of the LPSs) was further purified using high-performance anion-exchange chromatography (HPAEC) on a column (4 × 250 mm) of CarboPac PA100 ( Dionex) that was eluted at 1 ml min−1 with a linear gradient of 1-5% 1 m sodium acetate in 0.1 m NaOH over 50 min. Several fractions were obtained containing caryophyllose oligosaccharides, and one fraction that yielded L-glucuronic acid. Therefore, L-glucuronic acid was used as the reference compound to assign peaks in the LPSs. The sample was subsequently treated with 48% aqueous HF to remove phosphate groups and to degrade the O-specific polysaccharide, followed by extensive dialysis against water and lyophilization (53 mg, 13% of the LPSs). This material was reduced with NaBH4 (20–22 °C, 18 h), then dialyzed and lyophilized (50 mg, 12.5% of the LPSs), and de-N-acetylated with 4 m KOH as described previously (15). The product was applied to a column (50 × 1.5 cm) of Sephadex G-50 (Amersham Biosciences, Inc.), the resulting oligosaccharide fraction (46 mg, 11.5% of the LPSs) was further separated utilizing gel-permeation chromatography with a column (50 × 3 cm) of TSK-40 (Merck) in pyridine/acetic acid/water (8:20:1000, by volume). The main fraction was then subjected to analytical HPAEC eluted with a linear gradient of 13–16% 1 m sodium acetate in 0.1 m NaOH over 50 min, from which oligosaccharide 2, representing the complete carbohydrate backbone of the lipid A-core region (1 mg, 0.25% of the LPSs) was obtained.

General and Analytical Methods—Determination of Kdo, neutral sugars including the determination of the absolute configuration of the heptose residues, organic bound phosphate, absolute configuration of the hexoses, GLC, and GLC-MS were all carried out as described elsewhere (16–19). For methylation analysis of the core region, the LPSs (80 mg) were hydrolyzed in 2M methanolic HCl (85 °C, 4 h), carboxymethylated, acetylated, and analyzed by GLC-MS. In a third methylation analysis of the core region, the LPSs (10 mg) were methylated, and the sample was methanolized in 2 m methanolic HCl (85 °C, 45 min) and examined by GLC-MS.

RESULTS

Compositional Analyses—The determination of the GlcN and organic bound phosphate contents of the LPSs from B. caryophylli gave a molecular ratio of about 2:1:3, respectively, suggesting that only the lipid A moiety is substituted by phosphate groups in nonstoichiometric amounts. The determination of the organic bound phosphate content of the supernatant obtained from acetic acid hydrolysis of the LPSs (containing the core region and the O-specific polysaccharides) gave negative results. Thus, the core region is free of phosphate.

Isolation and Characterization of Oligosaccharide 1—Oligosaccharide 1 (Fig. 1) was isolated from the supernatant obtained by gel-permeation chromatography and HPAEC after hydrolysis of the LPSs in 1% aqueous acetic acid. Its compositional analysis revealed the presence of Kdo and heptose in a molecular ratio of ~1:1, and methylation analysis yielded the derivatives of one residue each of terminally linked heptose and 5-substituted Kdo. Methanalysis of the sample and acetylation followed by analysis by GLC-MS identified the disaccharide Hep-Kdo. The structure of oligosaccharide 1 was established by 1H and 13C NMR spectroscopy. Chemical shifts were assigned utilizing COSY, TOCSY, NOESY, and HMOC experiments (Table I). Because of its free reducing end, the Kdo residue was present as α- as well as β-pyranose; thus, two sets of signals were visible in the one-dimensional NMR spectra. Despite the similarity of the 13C chemical shifts to published data (29), the linkage of the heptose residue to O-5 of Kdo was proven by the downfield shifts of the signal for its C-5 (α-Kdo, 75.5 ppm; β-Kdo, 74.2 ppm) and the NOE connectivity between H-1 of the heptose and H-5 and H-7 of Kdo. To confirm the presence of the disaccharide in the LPSs, the LPSs were methylated and then mildly methanolysed. Analysis of the sample by GLC-MS gave ions at m/z 263, 291, and 351
Isolation and Characterization of Oligosaccharide 2—After dephosphorylation, reduction and deacylation of the LPSs from *B. caryophylli*, which destroyed the O-specific polysaccharides, oligosaccharide 2 (Fig. 1) was isolated by HPAEC. Its low yield was due to the fact that after treatment of the de-O-acylated LPSs with HF, followed by reduction and de-N-acetylation, a mixture composed of various higher oligo- and smaller polysaccharides containing sugar residues from the O-specific polysaccharides was present, which could be further separated by gel-permeation chromatography. From this, only one fraction contained oligosaccharide 2, which was finally purified by HPAEC. Its compositional analysis identified d-GlcN, d-Glc, d-Gal, Hep, GlcN-ol, and Kdo. Methylation analyses of the oligosaccharide yielded the derivatives of terminal Glc, terminal Gal, 6-substituted Glc, 2,6-disubstituted Glc, terminal Hep, 3,4-disubstituted Hep, 3,7-disubstituted Hep, 5-substituted Kdo, 4,5-disubstituted Kdo, 6-substituted GlcN, and 6-substituted GlcN-ol. No traces of an unsubstituted Kdo residue could be found. Additionally, GLC-MS analysis of the methanolized methylated oligosaccharide revealed the presence of the disaccharide Hep-Kdo (ions at *m/z* 263, 291, and 351 (J1 fragment)).

**NMR Spectroscopy of Oligosaccharide 2**—The structure of oligosaccharide 2 was established by 1H and 13C NMR spectroscopy. Chemical shifts were assigned utilizing COSY, TOCSY, NOESY, ROESY, HMQC, and HMQC-TOCSY experiments. Anomeric configurations were assigned on the basis of the chemical shifts observed, and J1,2 values, which were determined from the DQF-COSY experiment. The data are presented in Table II. The anomeric region of the 1H NMR spectrum (Fig. 2) contained 10 signals, representing four heptose, five hexose, and one hexosamine residues. Their identification was possible by the complete assignment of all signals and the determination of the J1,2H vicinal coupling constants. One hexose (residue L, see Fig. 1) and the hexosamine (B) residue possessed the β-altro configuration, which was supported by a NOESY experiment that yielded for both sugars intra-residual NOE connectivities from H-1 to H-3 and to H-5. Three hexoses (H, I, and M) possessed the α-altro, one hexose (K) the α-galacto, and the heptoses (E, F, G) the α-manno configuration. The characteristic signals of H-3 of two Kdo residues were present at 1.994 ppm (H-3a) and 2.081 ppm (H-3eq) (residue C) and 1.800 ppm (H-3ax) and 2.230 ppm (H-3eq) (residue D). Their α-configuration was established on the basis of the chemical shift of their 3eq proton and by measurement of the J1H,3eq and J2H,3eq coupling constants (30, 31). All these sugars were pyranoses. Finally, one residue of glucosaminol was identified, originating from dephosphorylated, reduced, and deacylated GlcN A.

The 13C NMR chemical shifts could be assigned by an HMQC experiment, using the interpreted 1H NMR spectrum. Ten anomeric signals were identified (Table I). The anomeric signals of
the Kdo residues C and D were not detected. Low field-shifted signals indicated substitutions at O-6 of residues A, B, and L, O-5 (D), O-3 and O-4 (E), O-4 and O-5 (C), O-3 and O-7 (F), and at O-2 and O-6 (H). G, I, K, M, and N were terminal sugars.

The sequence of the monosaccharide residues was determined using NOE data (Fig. 3). NOE contacts between anomeric and trans-glycosidic protons were observed for all hexose and heptose residues, and for GlcN B. An interresidual NOE contact was observed between H-1 of GlcN B to H-6a of GlcNol A, thus establishing the (1→6)-linkage of the lipid A backbone. Since Kdo possesses no anomeric proton, it was not possible to deduce the linkage of Kdo C to GlcN B by an NOE contact. However, since all other linkages of oligosaccharide 1 could be identified by NOE connectivities, the (2→6)-linkage of C to B could be established by the downfield $^{13}\text{C}$ chemical shift of C-6 of B (64.2 ppm, Table I). The deoxy protons H-3ax and H-3eq of Kdo C gave an NOE contact to H-6 of Kdo D (32), and H-3ax of C gave one to H-5 of heptose E. These interresidual NOE

**TABLE II**

$^1\text{H}$ and $^{13}\text{C}$ NMR chemical shifts (ppm) and coupling constants (in brackets) of sugar residues of the core-lipid A backbone (oligosaccharide 2) of LPS from B. caryophylli

Chemical shifts are expressed relative to acetone ($^1\text{H}$, 2.225 ppm; $^{13}\text{C}$, 34.5 ppm; at 27 °C): monosaccharides are as shown in Fig. 1.

| Sugar residue | H-1/C-1 | H-2/C-2 | H-3/C-3 | H-4/C-4 | H-5/C-5 | H-6a,b/C-6 | H-7a,b/C-7 | H-8a,b/C-8 |
|--------------|---------|---------|---------|---------|---------|-----------|-----------|-----------|
| Gal K        | 5.455   | 3.655   | 3.841   | 4.099   | 4.066   | 3.993     | 3.747     | 3.647     |
| (J 3.3 Hz)   |         |         |         |         |         |           |           |           |
| Hep IV N     | 5.296   | 4.031   | 3.889   | 3.862   | 3.765   | 3.993     | 3.747     | 3.647     |
| 97.9         | 69.9    | 70.3    | 70.5    | 72.5    | 63.0    |           |           |           |
| Glc I H      | 5.082   | 3.997   | 3.867   | 3.981   | 4.030   | 4.085     | 3.747     | 3.647     |
| 97.8         | 80.2    | 69.9    | 70.0    | 71.5    | 66.3    |           |           |           |
| Hep II F     | 5.082   | 4.071   | 4.222   | 3.894   | 3.763   | 4.175     | 3.727     |           |
| 102.5        | 70.7    | 77.7    | 66.5    | 72.0    | 69.9    | 70.0      |           |           |
| Hep I E      | 5.057   | 4.114   | 4.000   | 2.820   | 3.762   | 4.105     | 3.779     | 3.745     |
| 102.6        | 70.7    | 74.7    | 74.1    | 72.9    | 70.1    | 63.5      |           |           |
| Glc IV M     | 5.036   | 3.807   | 3.874   | 3.996   | 3.742   | 3.730     |           |           |
| (J 3.3 Hz)   | 98.4    | 68.9    | 69.8    | 70.5    | 72.5    | 61.3      |           |           |
| Glc II I     | 4.982   | 3.575   | 3.738   | 3.428   | 3.695   | 3.760     | 3.841     |           |
| (J 3.3 Hz)   | 98.4    | 72.0    | 70.9    | 72.0    | 72.4    | 60.9      |           |           |
| Hep III G    | 4.918   | 3.999   | 3.860   | 3.857   | 3.642   | 4.033     | 3.717     | 3.722     |
| 101.0        | 70.5    | 71.0    | 66.5    | 71.9    | 68.9    | 63.4      |           |           |
| Glc III L    | 4.610   | 3.310   | 3.497   | 3.437   | 3.659   | 3.972     | 3.830     |           |
| (J 7.7 Hz)   | 103.5   | 72.8    | 76.0    | 70.0    | 75.1    | 66.9      |           |           |
| Kdo I C      | 175.6   | 1.944/2.081 | 4.216 | 4.242 | 3.718 | 3.863  | 3.768/3.994 |           |
|               | 34.7    | 69.6    | 70.2    | 72.9    | 70.0    | 63.9      |           |           |
| Kdo II D     | 175.6   | 35.5    | 66.5    | 74.1    | 72.9    | 70.9      | 64.0      |           |
| GlcN B       | 4.464   | 2.760   | 3.391   | 3.691   | 3.500   | 3.505     | 3.609     |           |
| (J 8.0 Hz)   | 104.2   | 56.7    | 75.1    | 71.4    | 76.0    | 64.2      |           |           |
| GlcNol A     | 3.762/  | 3.540   | 3.832   | 3.564   | 4.107   | 3.622     |           |           |
| 3.873        | 59.3    | 55.8    | 68.9    | 71.8    | 71.8    |           |           |           |

**FIG. 2.** $^1\text{H}$ NMR spectrum of oligosaccharide 2. The spectrum was recorded at 600 MHz and 27 °C.
connectivities are characteristic for the sequence α-Hep-(1→5)-[(α-Kdo-(2→4))-α-Kdo]. Accordingly, a strong NOE contact between H-1 of E and H-5 of C, together with weak contacts to H-7 and H-8 a, b, was observed. NOE connectivities between H-3ax of Kdo D and H-3 and H-5 of Hep N demonstrated a close proximity between these two residues, and, accordingly, H-1 of N showed a strong NOE contact with H-5 of D, thus demonstrating that this heptose residue is linked to O-5 of Kdo D. H-1 of β-Glc L gave a strong NOE signal to H-4 of E and was substituted at O-6 by α-Glc M, as could be demonstrated by a strong NOE contact between H-1 of M and H-6a,b of L. Heptose E was substituted at O-3 by Hep F, as was established by an NOE contact between the H-1 of F and H-3 of E. Heptose F was substituted at O-7 by Hep G, which was proven by a strong NOE connectivity between H-1 of G and H-7a,b of F. Heptose F was substituted at O-3 by a 2,6-disubstituted α-glucose, residue H. This was inferred from the identified NOE contacts between H-1 of H and H-3 (strong) and H-4 (medium) of F. The H-1 protons of H and Gal K showed NOE contacts to H-2 of K and H, respectively. Furthermore, a NOE contact was found between these two anomeric protons, definitely proving the (1→2)-linkage of K and H. Accordingly, proton H-4 of residue F possessed a strong NOE connectivity to H-1 of Gal K, indicating a close proximity of these residues which owes to the (1→2)-linkage of K and H. Finally, Glc I was linked to O-6 of H, as indicated by an NOE contact between H-1 of I and H-6a,b of H.

The HMBC spectrum confirmed the major portion of the structure assigned for oligosaccharide 2, since it contained most of the required long range correlations to demonstrate the proximity of the residues. Together with intraresidual connectivities, the interresidual ones between H-1/C-1 of heptose N and C-5/H-5 of Kdo D, H-1/C-1 of E and C-5/H-5 of C, H-1/C-1 of L and C-4/H-4 of E, H-1/C-1 of F and C-3/H-3 of E, and between H-1/C-1 of residue H and C-3/H-3 of F were significant.

A MALDI-TOF mass spectrum of oligosaccharide 2 gave a molecular ion at m/z 2359.6 ([M + H]+), which characterized a molecule consisting of five hexose, four heptose, two Kdo, one heoxosamine, and one heoxosaminide residuals.

In summary, we have established the structure of the carbohydrate backbone of the core-lipid A region of the LPSs from B. caryophylli as depicted in Fig. 1.

**DISCUSSION**

*B. caryophylli* had been named *Pseudomonas caryophylli* before 1973 and, thus, was taxonomically included in the genus *Pseudomonas*, which, because of the diversity of functions found in its members, harbored a large number of species (1). Many attempts to develop systems of classification of *Pseudomonas* species had failed during the first half of the 20th century, and it was then the research on rRNA sequence similarities among *Pseudomonas* species that resulted in an internal subdivision of the genus into five RNA homology groups. This subdivision was largely confirmed by investigations on e.g. fatty acid compositions, the appearance of the outer membrane protein OmpP, and genome structure and organization. The first of the RNA homology groups (RNA group 1) contains authentic *Pseudomonas* species, and RNA group 2 consists of species of a new genus named *Burkholderia*. Quite a number of structures of LPSs from *Pseudomonas* and some from *Burkholderia* species have been investigated so far. With regard to the core region of LPSs, which is structurally more conserved than the O-specific polysaccharide, published structures indicate that the core regions of *Pseudomonas* LPSs differ from those of *Burkholderia* LPSs (10, 33), which is confirmed by the data presented in this paper. In particular, one residue of D-glycero-α-talo-2-olupyranosonic acid (Ko) was identified in the LPSs of *Burkholderia cepacia* and *Burkholderia pseudomallei*, replacing the branching Kdo (Kdo II). This has not been identified in any LPS from *Pseudomonas* and, thus, might be of chemotaxonomical importance for the differentiation of both genera.

The linkages of the sugars in the O-specific polymers of the LPSs of *B. caryophylli* are acid-labile, and in preliminary experiments it could be shown that treatment of the LPSs with 48% aqueous HF (4 °C, 48 h) not only removed the phosphate groups but also cleaved the O-specific polysaccharides. Thus, we applied this method and succeeded, after additional reduction and deacylation of the LPS, in the isolation of the complete carbohydrate backbone of the core-lipid A region. Its structure (Fig. 1) could be established from chemical and methylation analyses and from NMR spectroscopic and mass spectrometric investigations. The core region contains a structural element that commonly occurs in the *Salmonella* type core regions of enterobacterial LPS, e.g. from *S. enterica* or *E. coli*, namely α-d-GlcP-(1→3)-[α-α-d-Hep-(1→7)]-α-α-d-Hepp-(1→3)-α-d-Hepp-(1→5)-[α-Kdo-2→4]-α-Kdo-(2→10). The Glp residue of this moiety is substituted by two hexoses, i.e. Glcp and Galp, which is similar to several enterobacterial core structures. In dissimilarity to the *Salmonella* type core regions, the core region from LPSs of *B. caryophylli* is free of phosphate and contains a Glp residue that is β-(1→4)-linked to Hep E. The last structural element represents a characteristic feature of phosphate-deficient (e.g. *Yersinia enterocolitica*, *Proteus mirabilis*) or phosphate-free (e.g. *Klebsiella pneumonia*) core regions. In the core region of LPSs from *B. caryophylli* it is substituted at O-6 by another α-Glcp residue. The same disaccharidic substituent occurs also in the core region of LPS from *P. mirabilis* strain R110/1959 (34, 35). Most strikingly and identified for the first time, the core region of *B. caryophylli* LPSs possesses two 1-α-d-Hepp-(1→3)-α-Kdo-2→ moieties (N-D and E-C), one of which is linked to lip A and the other to Kdo C. In several cases, a (nonstoichiometric) substitution of the branching Kdo residue with other sugars has been identified. Despite the fact that in several LPSs this residue is substituted at O-4 (S. enterica, *E. coli*, *Chlamydia* (9, 36) or O-8 (Chlamydia (36)) by a third Kdo residue, it may carry a substituent at O-4 (d-GalpA in *Rhizobium etli* CE3 (37)), at O-5 (α-l-Rhap in *E. coli* K-12 (38); d-GalpA in *R. etli* CE3 (37); d-GlcP-(1→4)-d-GalpA disaccharide in *Ochrobactrum anthropi* (21)), at O-7 (α-l-Galp in *E. coli* R2 strain EH100 (39)) and at O-8 (β-1-Arap4N in *Legionella pneumophila* (40)).

With regard to these structures, a substitution of Kdo D at O-5 by Hep may be considered as just another variant. However, with regard to biosynthesis of LPS, this substitution is of...
higher bearing. Biosynthesis of the core region is best established for the LPSs of E. coli (11–13). It begins with the attachment of two Kdo residues to precursor IV, the tetraacylated and bisphosphorylated GlcN-disaccharide, which is performed by one Kdo-activator WaaA (KdaT). This step occurs differentially in LPS biosynthesis of Pseudomonas aeruginosa, where both Kdo residues are transferred to the completed (fully acylated) lipid A (41, 42). However, here and in E. coli, after completion of lipid A employing two additional acylation steps, the Kdo that is attached to lipid A (Kdo I, residue C in Fig. 3) is substituted at O-5 by Hepp, a step that is brought about by heptosyltransferase I, which is encoded by the gene waaC. In a next step of E. coli LPS biosynthesis, this Hepp residue is then substituted at O-3 by another Hepp through the action of heptosyltransferase II, which is encoded by the gene waaF. Then further steps of core biosynthesis follow, i.e., attachment of the first Glep (by waaG) and the third Hepp to Hep II (by waaQ), and completion of the outer core region. Possibly, decorations of the core, like the attachment of branching sugars or outer core heptose residues (e.g. Hepp in E. coli K12 (38), do-Hepp in K. pneumoniae (19) or P. mirabilis R110/1959 (34, 35), occur at later stages of the biosynthesis. With regard to the introduction of outer core heptose residues in LPS core biosynthesis, it is unknown whether the same heptosyltransferases that furnish the inner core region are utilized again or whether other, specific heptosyltransferases are activated. This is also true for the biosynthesis of the two 1→a-D-Hepp (1→5)-a-Kdo-2→ moieties in the LPSs of B. caryophylli, and current data do not favor one possibility over the other.

Several core structures of LPSs from Ps. aeruginosaa and Ps. fluorescens have been published (10); however, with the core structure of LPSs from B. caryophylli these only share the structural element 1→a-D-Hepp (1→3)-l→a-D-Hepp (1→5)→a-Kdo-2→. One of the distinguishing features of B. caryophylli LPSs is their low phosphate content. Of LPSs from the genus Burkholderia, two core structures are known, i.e., from LPSs of B. cepacia GIFU 645 (43) and from B. pseudomallei GIFU 12046 (33). Both structures are similar to that of the core region from B. caryophylli, since they are free of phosphate and possess the structural element 1→a-D-Hepp (1→7)-l→a-D-Hepp (1→3)-β-g-D-Glc (1→4)-l→a-D-Hepp (1→5)→a-Kdo-2→. However, in these cases Kdo I is substituted at O-4 by v-glycéro-o-α-talo-oct-2-ulopyranosonic acid (Ko) rather than Kdo. In the LPSs of B. caryophylli, only small amounts of Ko could be detected, and no core oligosaccharide possessing this sugar could be isolated.2 It is thus unclear whether the same α-Ko (2→4)-α-Kdo disaccharide is present in this core region. Whereas in the core region of B. pseudomallei Hep II is substituted at O-3 by α-D-Glc, this particular Hep carries in the core region of LPS from B. cepacia an l-Rhap residue at O-2, which represents another unusual structural feature.

Acknowledgments—We thank Regina Engel for technical assistance, Hans-Peter Cordes for recording the NMR spectra, Angela Amoresano for recording the MALDI-TOF mass spectrum, Hermann Möll for help with GC-MS, and Yasunori Ishikiy for valuable discussions.

REFERENCES

1. Palleroni, N. J. (1993) Antonie Leeuwenhoek 64, 231–251

2. Jones, L. K. (1941) Phytopathology 31, 199

2 A. Molinaro, C. De Castro, C. Lanzetta, M. Parrilli, and O. Holst, unpublished data.