

Otto Holst

2.1 Introduction

Most Gram-negative bacteria (exceptions are *Treponema pallidum*, *Borrelia burgdorferi* and *B. hispanica*, *Sphingomonas capsulata* and *S. paucimobili*, *Thermus thermophilus*, and *Meiothermus taiwanensis* [1–7]) contain lipopolysaccharide (LPS) in their outer membrane [8]. In general, the LPS may be present either in the smooth form (S, possessing the polysaccharide region) or in the rough form (R, lacking the polysaccharide, also called lipooligosaccharide, LOS). Both forms contain lipid A [9] and a core oligosaccharide (OS) that comprises up to 15 sugar residues [8–17]. In the S-form LPS, the core OS region is substituted by a polysaccharide, which most often is an O-specific polysaccharide (O-antigen, for structures see Chap. 3), and in other cases is the enterobacterial common antigen (only in *Enterobacteriaceae* [18]) or a capsular polysaccharide [19]. For a long time a dogma existed claiming that mutants of LPS-containing Gram-negative bacteria are not viable without a minimal core OS structure. However, viable mutants synthesizing only lipid A or a precursor thereof have been recently isolated [20, 21].

2.2 Historic Outline

Richard Pfeiffer identified endotoxin in 1892 [22]. However, it took some 60 years to establish the appropriate extraction protocol (the hot phenol–water procedure [23, 24]) by which the isolation of rather pure endotoxin was possible. It became

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clear soon after that endotoxin consists of sugars, phosphates, and fatty acids, and is a lipoglycan termed “lipopolysaccharide” [25]. However, it was also shown that LPS purity depends on the method of extraction. Purified LPS from *Salmonella* displayed endotoxic and antigenic activities. The former could be assigned to the lipid moiety and the latter to the polysaccharide, which was also called O-antigen. At that time, researchers were convinced that LPS was simply built up from only a lipid and a polysaccharide (the lipid was later named lipid A since also a second lipid, lipid B [26], and a third one were identified; but it turned out that the latter two were not part of LPS and thus of no importance here). In the early 1960s, it had become clear from analyses of samples extracted from *Salmonella* that LPS possessed two classes of sugars: common ones and those that occurred only in particular LPS [27]. Consequently, a working hypothesis was generated claiming that all *Salmonella* LPS should possess a common carbohydrate core substituted by the O-antigen.

The structure of a complete LPS molecule was thought to be much more complex than it is known today [28, 29]. The lipid A was believed to consist of a poly-(*N*- β -hydroxymyristoyl-D-glucosamine phosphate), which is substituted by ester-linked long-chain fatty acids and, via 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) [30] at a then unknown position, by heptose phosphate chains via phosphodiester bridges (Fig. 2.1). These chains in turn are substituted by short OSs of hexoses and GlcN as well as by the O-antigen consisting of repeating units. Thus, the LPS was proposed to possess a highly polymeric branched comb-like architecture. This overall structure was revised at the turn of the decade, and in 1971, a general LPS architecture was reported, which is still valid for most LPS today [31]. At that time, newer data had indicated that lipid A is a phosphorylated and acylated GlcN disaccharide. However, phosphodiester bridges between these units could still not be excluded. This was also the case concerning the core region but its general structure had become that of an OS.

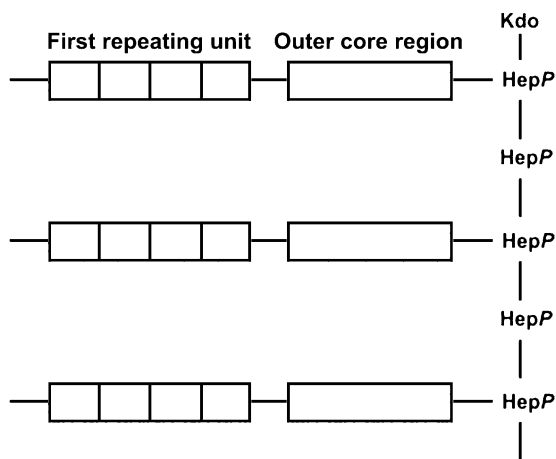


Fig. 2.1 The architecture of the core-O-antigen regions proposed in 1966 [28, 29]

The basis for this progress was the availability of a variety of rough (R)-type mutants, first obtained from *Salmonella* [29]. Also, a new method for the extraction of particularly R-type LPS was invented [32]. All R-mutants synthesized only short-chain LPS (LOS) comprising lipid A and the core region or a part thereof. They are very helpful for the detailed structural analysis of the enterobacterial LPS core region, which still holds true to date. Investigations on such LPS had been performed already in the early 1960s and led to the identification of an RI and an RII core, which were later re-named Rb and Ra (the complete core), respectively. The Ra-mutant was defective in O-antigen biosynthesis, and the Rb mutant additionally in core biosynthesis. Other mutants with shorter core regions were also identified and termed Rc through Re [28]. Later, additional *Salmonella* mutants (named Rd₁ and Rd₂) and mutants obtained from other bacteria, like *Escherichia coli* and *Citrobacter*, were obtained and their LPS investigated (summarized in Ref. [30]). Also, so-called SR-mutants were available which possessed in their LPS a complete core region plus one repeating unit; thus, the linkage position of the O-antigen at the core region could be identified.

In the following years, significant progress concerning the structural analysis of the core regions of LPS from *Salmonella* (one core type) and *E. coli* (in which five core types were identified, R1–R4 and K-12) could be achieved (summarized in Ref. [33]). It was proposed that the core OS could be subdivided into inner (containing Kdo and heptose) and outer (consisting of hexoses and hexosamines) regions. However, no complete structures were available due to difficulties in chemical analyses of the core region at that time (see below). Also, it had been proposed that there were LPS that contained no Kdo, like that of *Vibrio cholerae*. This was revised later when the presence of Kdo-4-phosphate was identified in this LPS, due to which the photometric determination of Kdo had failed in earlier studies.

From the mid-1980s on, owing to improved analytical tools and methods, including modern nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), and to development of protocols, which e.g. brought about the isolation of a complete lipid A-core backbone, a lot of core structures from a range of bacterial species have been reported (summarized in Ref. [8–17]; see also below). A comparison of these structures clearly verifies the hypothesis of the 1960s, which proposed a broad variety of core OS structures. It is clear now that all LPS molecules contain at least one Kdo residue (which in some LPS may to a certain extent be replaced by D-glycero-D-talo-oct-2-ulosonic acid, Ko) and that not all core regions contain heptose or phosphate residues and/or not all may be subdivided into inner and outer core.

2.3 Structural Analysis of the Core OS Region

Owing to (1) the difficult chemistry of Kdo and the phosphate substitution, (2) the lack of procedures for the isolation of highly purified OSs, and (3) limitations in the application of NMR spectroscopy and MS, the structural analysis of the core region

could not be completely elucidated for quite some time. This was improved since the late 1980s when an appropriate protocol for the determination of Kdo was developed (reviewed in Ref. [34]). However, a full analysis of the phosphate substitution still remains problematic.

The current analytical strategy includes the investigation of the isolated and purified LPS by MS prior to the application of any degradation protocol. This provides valuable information on the LPS composition. Apart from matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS [35], highly resolved spectra have been obtained from electrospray ionization (ESI) MS [36], also in combination with capillary electrophoresis of either O-deacylated LPS [37] or native LPS or lipid A [38]. A degradation pathway to obtain phosphorylated OSs from LPS [39] was developed, which utilizes successive mild hydrazinolysis and 4 M KOH treatment followed by isolation of pure compounds by high-performance anion-exchange chromatography. In the recent years, this protocol has been applied by several investigators and has helped characterizing a variety of core OS regions from various bacteria.

This approach has some limitations in the presence of phosphodiester, diphosphate, diphosphodiester, acetyl and carbamoyl groups, which are components of many LPS and are readily cleaved under strong alkaline conditions, making it impossible to identify their positions in the core OS. Also, the alkaline treatment leads to phosphate migration in the case of substitution by 2-aminoethyl phosphate, and to β -elimination of 4-substituted uronic acid residues [39]. In some cases, the O-deacylated product obtained after mild hydrazinolysis can be analyzed by NMR spectroscopy particularly, using ^{31}P , ^1H -correlated experiments, which helps to demonstrate the presence and location of phosphodiester, diphosphate, and diphosphodiester groups. However, in other cases spectra may be poorly resolved and NMR spectroscopy is not applicable. MS may help to identify the sugar residue that is substituted but the identification of the linkage position may still hardly be possible.

It is recommended to employ (in addition) the “traditional” protocol to isolate the core region, namely mild acetic acid (buffer) hydrolysis in order to cleave the linkage between the core region and lipid A [31, 40]. This alternative approach is applicable in any case and not only if a deacylated product cannot be obtained (e.g., when the anomeric position of the lipid A backbone is not substituted) or the deacylation procedure results only in a partial structure (e.g., due to β -elimination of a 4-substituted uronic acid). In a variant, the addition of 1% sodium dodecyl sulfate improves the cleavage [41]. Two other protocols have been described, using either a mixture of isobutanoic acid and 1 M ammonium hydroxide (5:3, v:v) [42] or triethylamine citrate [43].

From S-form LPS, usually a mixture of an O-antigen-core polysaccharide and a core OS is obtained, and sometimes investigators have the good luck to yield in addition an SR-OS fraction. Such mixture can be routinely separated by gel-permeation chromatography, e.g. on a column of Sephadex G-50 eluted with a pyridine-acetic acid buffer.

From R-form LPS (LOS), mild acid hydrolysis yields (an) incomplete core OS(s), since if branched Kdo-saccharide is present, the branching Kdo residue(s) is/are also cleaved. Thus, the structure of this moiety cannot be determined. Also, Kdo residues that may be present at the nonreducing terminus of the core (like in *Klebsiella pneumoniae* [44]) are cleaved and structural information is lost. Still, products can be isolated that represent the major part of the core region. With regard to the substitution pattern, phosphodiester, diphosphate, diphosphodiester, acetyl and carbamoyl groups are at least in part retained (see for example Refs. [45, 46]). The isolation of pure phosphorylated compounds can be achieved by high-performance anion-exchange chromatography utilizing a gradient of sodium acetate in water at pH 6 [39].

Prior to any degrading chemical analysis, pure OSs are extensively investigated by NMR spectroscopy, applying in particular homonuclear and heteronuclear two-dimensional experiments. Also, small amounts (10–20 µg) are used for MS studies. In chemical analysis of LPS as well as the O-antigen and the core region (the “wet chemistry” approach [47]), investigators have to deal with different sugars possessing different stability under acidic hydrolysis conditions. Routine hydrolysis protocols comprise e.g. the use of 0.1 M HCl (100°C, 48 h) or trifluoroacetic acid (2 M, 120°C, 2 h, or 4 M, 100°C, 4 h). After such hydrolyses followed by reduction and acetylation, hexoses and pentoses are identified by GLC. However, more stable linked amino sugars are identified only in small amounts and acid-labile compounds like Kdo and dideoxy sugars are largely destroyed.

To get an idea about which sugars are present in the core region, it is recommended to begin with two different methanolysis protocols, e.g. analysis of acetylated methyl glycosides after mild (0.5 M methanolic HCl, 85°C, 45 min) and strong (2 M methanolic HCl, 85°C, 16 h) methanolysis conditions. Here, it is possible to detect deoxyhexoses, hexoses, pentoses, uronic acids, amino sugars, and Kdo as well as fatty acids of lipid A. After that, appropriate conditions for the production of alditol acetates can be chosen for the identification and quantification of different monosaccharides. To determine the substitution pattern of the monosaccharide residues, methylation analysis is performed, utilizing either the Hakomori methylation protocol [48] or that developed by Ciucanu and Kerek [49]. Particular protocols on methylation analysis of the Kdo region have been published [50]. If uronic acids are present, these are esterified and the methyl ester function is reduced prior to the hydrolysis step. Samples are finally analyzed by GLC-MS.

2.4 General Structural Features of the Core Region

The chemical structures of the core regions of bacterial LPS have been regularly and extensively summarized since 1992 [8–17]. Therefore, mostly general features of core structures are described here.

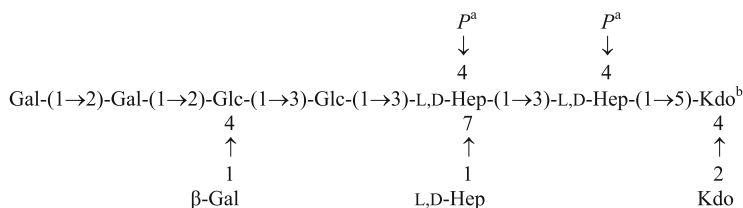
All core region chemical structures identified so far are less varied than those of O-antigens. Still, only one structural element is present in all core regions, namely that Kdo residue which links the core to the lipid A. In many bacteria, the core region contains *L-glycero-D-manno-heptose* (L,D-Hep) and an L- α -D-Hep-(1 \rightarrow 3)-L- α -D-Hep-(1 \rightarrow 5)-[α -Kdo-(2 \rightarrow 4)]- α -Kdo tetrasaccharide (Hep II, Hep I, Kdo II, and Kdo I, respectively), which may be further substituted by other sugars or phosphate residues, or sometimes by acetyl groups or amino acids. In addition to L,D-Hep, several LPS contain its biosynthetic precursor, *D-glycero-D-manno-heptose* (D,D-Hep). There are other LPS that contain only D,D-Hep or even lack any heptose. Either Kdo I (in *Acinetobacter*) or Kdo II (in Burkholderiaceae, *Yersinia pestis*, and *Serratia marcescens*) may be replaced by the stereochemically similar sugar acid Ko, the biosynthesis of which and regulation of the exchange between Kdo and Ko have not been elucidated so far.

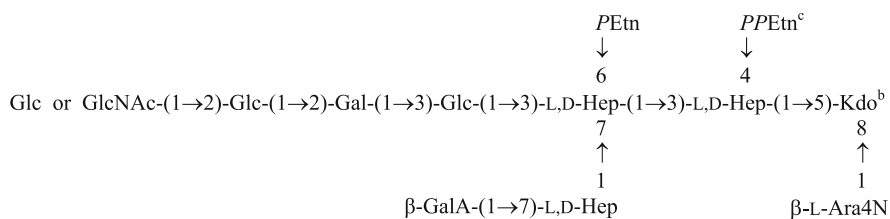
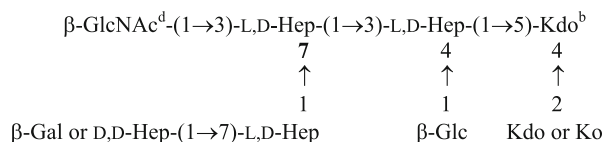
2.5 Core Structures of Some Important Pathogenic Bacteria

2.5.1 Enterobacteriaceae

Two types of enterobacterial core regions have been recognized, namely the *Salmonella* type core and the core different from the *Salmonella* type. The first is characterized by the common structural element L- α -D-Hep-(1 \rightarrow 7)-L- α -D-Hep-(1 \rightarrow 3)-L- α -D-Hep-(1 \rightarrow 5)-Kdo substituted at O-3 of Hep II by glucopyranose. Hep I and II are phosphorylated and O-4 of Hep I is not substituted by a saccharide. In the second core type, the same common partial structure is present but lacks Glcp at O-3 of Hep II, the heptose residues are not generally phosphorylated, and Hep I is substituted by a hexose residue or an OS at O-4. The core regions of LPS from *E. coli*, *Providencia* and *Yersinia* species are shown as examples. Those of *Providencia* and *Yersinia* occur as two glycoforms differing in sugar residues in the outer regions (D-Glcp vs. D-GlcpNAc or β -D-Galp vs. D- α -D-Hep, respectively).

Escherichia coli R4 (*Salmonella* type) [51]



Providencia rustigianii O-34 (*Salmonella* type) [52]*Yersinia pestis* (other than *Salmonella* type), various strains [53]

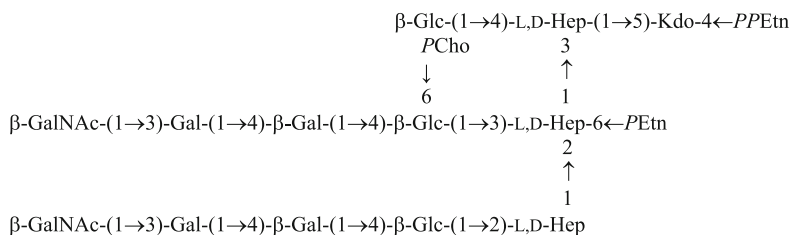
Here and below where not stated otherwise, sugars are α -D-pyranosides. Abbreviations: *PEtn*, 2-aminoethyl phosphate; *PPeTn*, 2-aminoethyl diphosphate; L-Ara4N, 4-deoxy-L-arabinose. ^aProduct obtained after *O*-deacylation. Further substituents are not known. ^bThis Kdo residue links the core region to lipid A. ^cEither *PPeTn* or *P* is present at this position. ^dNon-stoichiometric substitution.

2.5.2 Pasteurellaceae

A high number of core structures have been reported for LPS of the genera *Haemophilus*, *Histophilus* and *Pasteurella*.

The core region of LPS from *Haemophilus influenzae* possesses as common partial structure the L- α -D-Hep-(1 \rightarrow 2)-[*PEtn* \rightarrow 6]-L- α -D-Hep-(1 \rightarrow 3)-[β -D-Glcp-(1 \rightarrow 4)]-L- α -D-Hep-(1 \rightarrow 5)-[*PPeTn* \rightarrow 4]- α -Kdo saccharide. Only this one Kdo residue is present which links the core region to lipid A. Other substitutions occur mainly at β -D-Glcp and Hep III. In particular, core regions of non-typeable *H. influenzae* strains have been investigated in the past years [9, 17].

Haemophilus influenzae strains 1200, 1268 [54]



Abbreviation: *PCho*, 2-trimethylammonioethyl phosphate (phosphocholine).

Haemophilus influenzae [55–59]

General structure $R^1 \rightarrow 6) - [R^2 \rightarrow 4)] - \beta\text{-Glc}^a - (1 \rightarrow 4) - L,D\text{-Hep}^b - (1 \rightarrow 5) - Kdo^c - 4 \leftarrow R^3$

3

↑

1

$R^4 \rightarrow 2) - L,D\text{-Hep}^d - (1 \rightarrow 2) - L,D\text{-Hep} - 6 \leftarrow P\text{Etm}$

3

↑

R^5

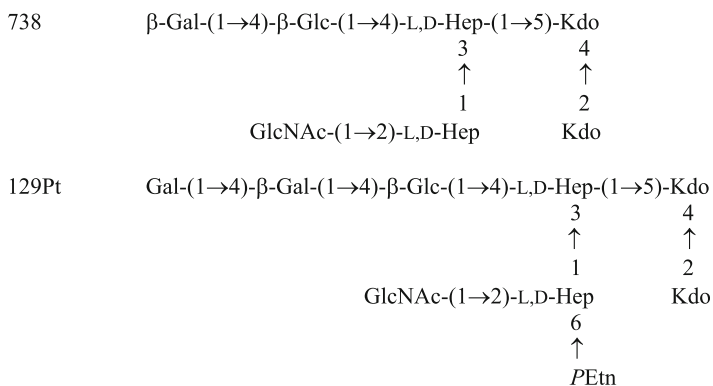
Strain

1003	R^1 <i>PCho</i>	R^2 OAc	R^3 <i>PEtm</i>	R^4 Neu5Ac-(2→3)-β-Gal-(1→4)-β-GlcOAc-(1	R^5 OAc
RM118	R^1 <i>PCho</i>	R^2 Neu5Ac-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1	R^3 <i>PEtm</i>	R^4 Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1	R^5 H
162	R^1 <i>PCho</i>	R^2 H	R^3 n.d.	R^4 β-GalNAc-(1→3)-β-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1	R^5 H
981	R^1 <i>PCho</i>	R^2 H	R^3 n.d.	R^4 β-GalNAc-(1→3)-β-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1	R^5 H
Form 1	R^1 <i>PCho</i>	R^2 H	R^3 n.d.	R^4 β-GalNAc-(1→3)-β-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1	R^5 H
Form 2	R^1 <i>PCho</i>	R^2 H	R^3 n.d.	R^4 β-GalNAc-(1→3)-β-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1	R^5 H
723	R^1 <i>PCho</i>	R^2 OAc	R^3 n.d.	R^4 H	R^5 <i>PEtm</i>

^aO-Acetylated at position 3 in strain 723. ^bO-Acetylated at position 2 in strain 723. ^cThis Kdo residue links the core region to lipid A. ^dO-Acetylated at unknown position in strain 723.

In LPS of *Histophilus (Haemophilus) somnus*, the core region comprises the α -Kdo-(2 \rightarrow 4)- α -Kdo disaccharide and the common L- α -D-Hep-(1 \rightarrow 3)-[β -D-Glcp-(1 \rightarrow 4)]-L- α -D-Hep-(1 \rightarrow 5)- α -Kdo structure. The β -D-Glcp residue is further substituted and Hep II carries either α -D-GlcpNAc or β -D-Galp at O-2. Hep II may contain one or two PEtN residues, and N-acetylneuraminic acid may be incorporated which is important for serum resistance and reduction of antibody binding. Two examples are shown below.

Histophilus somnus [60, 61]

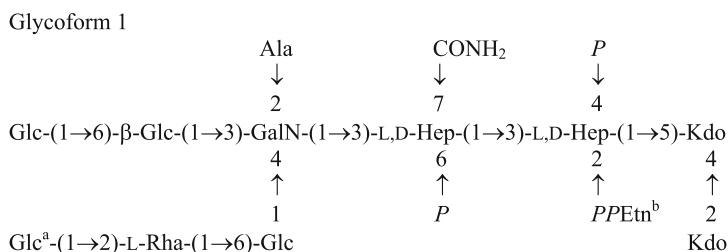


The core region of the LPS from *Pasteurella multocida* has as a common feature of the L- α -D-Hep-(1 \rightarrow 2)-L- α -D-Hep-(1 \rightarrow 3)-[β -D-Glcp-(1 \rightarrow 4)]-[α -D-Glcp-(1 \rightarrow 6)]-L- α -D-Hep-(1 \rightarrow 5)- α -Kdo hexasaccharide [9]. In strain VP161, both α -Kdo-(2 \rightarrow 4)- α -Kdo disaccharide and α -Kdo-4 \leftarrow PPEtN are present [62, 63].

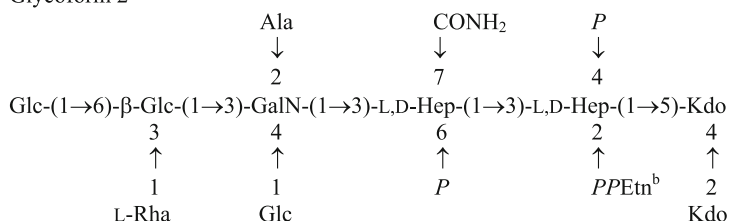
2.5.3 Pseudomonadaceae

In the core of LPS of *Pseudomonas aeruginosa*, a α -D-GalpN residue is present which in most strains studied is N-acylated by L-alanine. It substitutes O-3 of Hep II of the L- α -D-Hep-(1 \rightarrow 3)-L- α -D-Hep-(1 \rightarrow 5)-[α -Kdo-(2 \rightarrow 4)]- α -Kdo tetrasaccharide, in which Hep II is further substituted at O-7 by a carbamoyl group. In all strains studied so far, the core region is highly phosphorylated. The outer region occurs as two glycoforms and is randomly O-acetylated.

Pseudomonas aeruginosa [45]



Glycoform 2

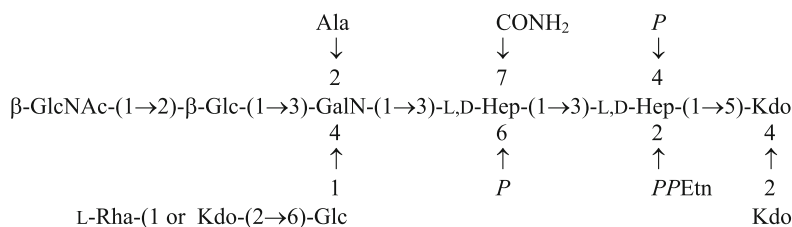


O-Acetylation is not shown. ^aThis glucose residue is a non-oligotary component. ^bEither PPEtn or P is present at this position.

In a *wbjE* mutant of *P. aeruginosa* PA103 (serogroup O-11), the full glycoform 1 core contains GalpNAc in place of GalpNAc and two or three phosphate groups as mono- or di-phosphates but no PEtn [64].

The inner core region of *Pseudomonas syringae* is similar to that of *P. aeruginosa*, whereas the outer region is different but also occurs as two glycoforms, one of which contains Kdo.

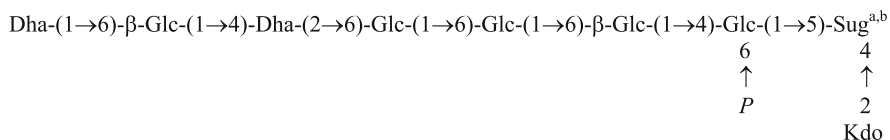
Pseudomonas syringae pv. *phaseolicola* [65]

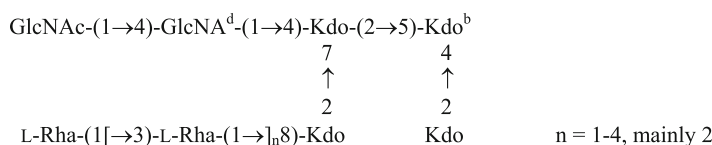
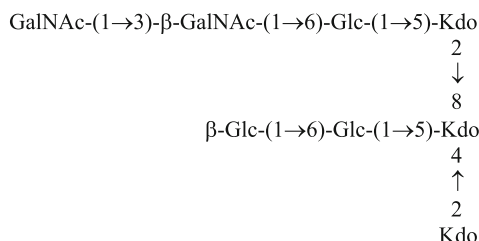


2.5.4 Moraxellaceae

Various core structures have been identified in the past in this family, either possessing Ko or not. Only one novel core region was identified recently, namely that of the LPS from the allergy-protective bacterium *Acinetobacter lwoffii* F78 [66]. Unexpectedly, it possesses the α-Kdo-(2 → 8)-α-Kdo disaccharide which was found earlier only in the core regions of LPS from the genera *Chlamydia*/*Chlamydophila* [12].

Acinetobacter haemolyticus ATCC 17906 [67]

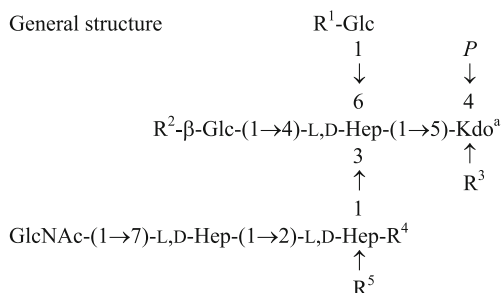


Acinetobacter baumannii NCTC 10303 [68]*Acinetobacter lwoffii* F78 [66]

Abbreviations: Dha, 3-deoxy-D-lyxo-heptulosaric acid; GlcNA, 2-amino-2-deoxy-D-glucuronic acid. ^aSug stands for Kdo (minor) or Ko (major). ^bThis Kdo residue links the core region to lipid A.

2.5.5 Vibrionaceae

Several structures of LPS core regions from *Vibrio cholerae* have been established [8–13]. They have as common feature one Kdo residue phosphorylated at O-4 and substituted at O-5 by the α -D-GlcpNAc-(1 \rightarrow 7)-L- α -D-Hep-(1 \rightarrow 2)-L- α -D-Hep-(1 \rightarrow 3)-L- α -D-Hep tetrasaccharide, in which Hep I is substituted by β -Glc at O-4 and α -Glc at O-6. Variations in core structures occur by different substituents at O-6 of both Glcp residues and Hep II as well as at O-2 or O-4 of Hep III. In all characterized structures, a β -linked fructose or sedoheptulose (D-*altro*-hept-2-ulose) residue is present at O-6 of the β -linked Glcp.

Vibrio cholerae [8–13]

^aThis Kdo residue links the core region to lipid A. R¹ may be H or Glc-(1 \rightarrow 6), R² D-fructofuranose or D-sedoheptulofuranose (Sedf), R³ H or PEtn \rightarrow 7, R⁴ H or L,D-Hep-(1 \rightarrow 6), R⁵ H or O-specific polysaccharide (OPS); in strain H11, R⁵ is OPS-(1 \rightarrow 4)- β -Sedf-(2 \rightarrow 3)- β -Gal-(1 \rightarrow 3).

Several LPS core regions of the genus *Aeromonas* have also been investigated [69–71]. For example, *A. salmonicida* ssp. *salmonicida* comprises four L- α -D-Hep residues, of which Hep I is α -(1 \rightarrow 5)-linked to Kdo-4-phosphate and in turn carries the L- α -D-Hep-(1 \rightarrow 6)- α -D-Glcp disaccharide at O-4 [69]. The heptose residue of this unit is differently substituted at O-4, resulting in heterogeneity. In addition to these four L- α -D-Hep residues, the core region of *A. hydrophila* AH-3 contains a D- α -D-Hep disaccharide substituting O-6 of β -D-Glcp at O-4 of Hep I [70].

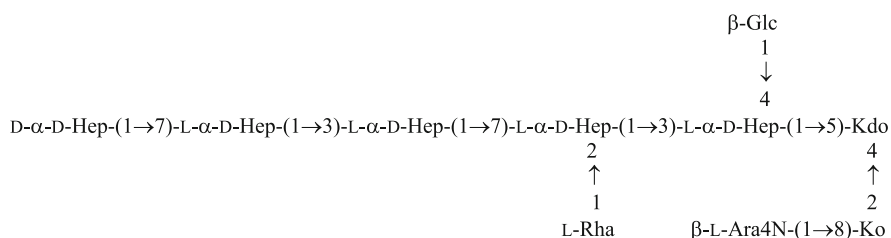
2.5.6 Burkholderiaceae

Bacteria of the genus *Burkholderia* possess LPS core structural features of chemotaxonomic value [72, 73]. The core regions that have been identified so far include in various amounts both α -Kdo-(2 \rightarrow 4)- α -Kdo and α -Ko-(2 \rightarrow 4)- α -Kdo disaccharides. The common partial structure is L- α -D-Hep-(1 \rightarrow 7)-L- α -D-Hep-(1 \rightarrow 3)-[β -D-Glcp-(1 \rightarrow 4)]-L- α -D-Hep-(1 \rightarrow 5)- α -Kdo.

In the LPS core of *Burkholderia caryophylli*, O-3 of Hep II is substituted by a branched glycan to which one of the O-specific polysaccharides (caryophyllan or caryan) is linked [73]. This core region possesses as a unique feature two L- α -D-Hep-(1 \rightarrow 5)- α -Kdo moieties.

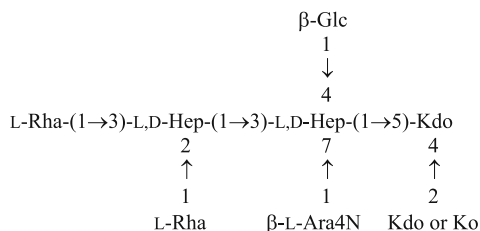
The core region of the LPS of *Burkholderia pyrrocinia* contains as a peculiar feature a linear heptose pentasaccharide consisting of four L_D-Hep and one terminal D_D-Hep residues.

Burkholderia pyrrocinia BTS7 [74]



B. pyrrocinia shares the α -L-Rha-(1 \rightarrow 2)-L- α -D-Hep-(1 \rightarrow 3)-[β -D-Glcp-(1 \rightarrow 4)]-L- α -D-Hep-(1 \rightarrow 5)-[-Ko-(2 \rightarrow 4)]- α -Kdo hexasaccharide with *Burkholderia cepacia* [71] and *Ralstonia solanacearum* [75]. The latter differs from representatives of the genus *Burkholderia* in the attachment of β -L-Arap4N to Hep I rather than Ko.

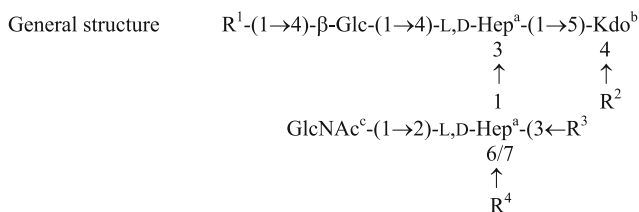
Ralstonia solanacearum Toudk-2 [75]



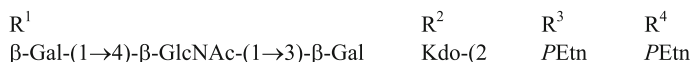
2.5.7 Neisseriaceae

In general, neisserial LPS are of the rough type. Thus, LOS and core regions consist of the L- α -D-Hep-(1 \rightarrow 3)-L- α -D-Hep-(1 \rightarrow 5)-Kdo trisaccharide, which is substituted by β -D-Glcp at O-4 of Hep I and by α -D-GlcpNAc at O-2 of Hep II. Structural variations occur by different substituents at O-4 of β -D-Glcp and at Hep II.

Neisseria [10]



Neisseria meningitidis M986 [76]



^aThe absolute configuration was not determined in *N. gonorrhoeae*. ^bThis Kdo residue links the core region to lipid A. ^cNon-stoichiometric O-acetylation (~0.5) occurs at O-3 of this residue.

Six LPS serotypes have been identified in the causative agent of gonorrhoea, *N. gonorrhoeae* [77]. The resistance of *N. gonorrhoeae* in patients and when grown in serum-containing media against the bactericidal effects of complement may be caused by sialylation of the LPS which occurs at a terminal β -D-Galp residue (1 \rightarrow 4)-linked to α -D-GlcpNAc. A further interesting observation is that phase variation of LPS caused by a different degree of sialylation controls both entry of *N. gonorrhoeae* into human mucosa cells (low degree of sialylation) and the resistance to complement-mediated killing (high degree of sialylation).

N. meningitidis is a causative agent of severe human diseases, as sepsis and meningitis. It is differentiated into 12 LPS serotypes [77], of which 8 possess lacto-N-neotetraose β -Galp-(1 \rightarrow 4)- β -GlcpNAc-(1 \rightarrow 3)- β -Galp-(1 \rightarrow 4)- β -Glcp. This common partial structure is identical to the glycosyl moiety of lactoneotraglycosylceramide, which is present on the membrane of human erythrocytes and thought to represent a mimicry antigen that helps to evade the host defense. Sialylation occurs also in some meningococcal LPS where α -Neu5Ac is (2 \rightarrow 3)-linked to the terminal Galp residue of lacto-N-neotetraose.

Other substituents of the core structures have been reported, e.g. two PEtn residues at Hep I in *N. meningitidis* strain BZ157 *galE* [78], glycine on O-7 of Hep II in various immunotypes [78, 79], and an O-acetyl group may be present at O-3 of GlcpNAc [80]. It has been proven that PEtn is located at O-6 of Hep II [81].

The considerable structural heterogeneity has been profiled by MALDI MS and related to cytokine induction [82].

2.6 Conclusions

To date, after more than 60 years of intensive structural research, a rather high number of core structures from LPS of various bacterial species have been elucidated. The general principle consists of a negatively charged core region (provided by phosphoryl substituents and/or sugar acids like Kdo and uronic acids), which strengthens the rigidity of the Gram-negative cell wall through intermolecular cationic cross-links.

The linkage of the core region to lipid A occurs always via a Kdo residue. However, in *Acinetobacter* Ko may replace Kdo non-stoichiometrically at this position. Also, there are core structures containing and lacking heptoses. In the first type, L,D-Hep or D,D-Hep alone, or both may be present. When present, D,D-Hep either decorates the inner core region (e.g. in *Yersinia*) or is attached to more remote parts of the carbohydrate chain. Furthermore, many core regions possess as partial structure the L- α -D-Hep-(1 \rightarrow 3)-L- α -D-Hep-(1 \rightarrow 5)- α -Kdo trisaccharide, and a genus often contains in its LPS a common core structural theme that varies by certain (non-)carbohydrate substituents.

Although mutants of *E. coli* K-12 were isolated that are viable possessing only lipid IV_A in their cell envelope, the common structural principle of LPS may still be described as core OS plus lipid A. Any (O-specific) polysaccharide expression in LPS is not a prerequisite for bacterial survival; still, the finding that the polysaccharide portion in S-form LPS may be furnished either by the O-chain or a capsular structure or the ECA clearly indicates that this LPS form is highly advantageous for many species.

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