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Bacterial lipopolysaccharides

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Abstract - Lipopolysaccharides are the O-antigens and the endotoxins of Gram-negative bacteria. They are localized in the outer membrane of the bacterial cell wall and play an important role in the pathogenicity of bacterial infections, as well as in interaction with the host and its defense system.
Lipopolysaccharides share a common architecture. They are built by a hydrophilic polysaccharide region which shows high structural variability and by a much less variable hydrophobic lipid moiety, termed lipid A, which anchors the molecule in the outer membrane of the cell wall. Lipid A is responsible for the pathophysiological properties of the so-called endotoxins.
Recent work has shown that a number of non-toxic and structurally deviating lipid A types occur in non-enteric bacteria and their structural or compositional pecularities were shown to be correlated in many instances with the phylogenetic position of the respective species, as evidenced by 16S rRNA homologies.

INTRODUCTION

Lipopolysaccharides (LPS) are a family of structurally related amphiphilic (macro-) molecules which share the same general architecture. They are essential constituents of the cell walls of almost all Gram-negative bacteria and of some cyanobacteria (ref. 1-3). Here they are exclusively localized in the outer leaflet of the outer membrane (ref. 4,5). With immuno-cytochemical methods, e.g. with ferritin- or gold-labeled specific antibodies and by using the "whole mount" technique, one could demonstrate that LPS is evenly distributed all over the entire bacterial surface (ref. 6).
Lipopolysaccharides are the main heat-stable antigens of the bacterial cell and as their so-called O-antigens have been of utmost importance for the classification (serotyping) of many Gram-negative species (ref. 7). Bacteria, invading a higher organism, are recognized by the body's defense system due to these distinct surface antigens and antibodies are subsequently produced directed against epitopes or determinants being embedded in or being part of the species-specific carbohydrate chain of the O-antigenic lipopolysaccharides.
The antibodies, directed against these O-specific determinants, are opsonic and bactericidal, and they may even protect against subsequent infections with antigenically related pathogens (ref. 2,8).
It was recognized almost a hundred years ago that material released from desintegrating bacteria causes a variety of pathophysiological effects and this material was therefore termed endotoxin by R. Pfeiffer (ref. 2,8), to discriminate it from exotoxins, known to be excreted by a number of important pathogenic bacteria.
The pathophysiological effects caused by endotoxins, such as fever, changes in the white blood cell count, disseminated intravascular coagulation, or -with higher doses- even irreversible shock and death, can be elicited also with highly purified isolated lipopolysaccharides, indicating that they are carrying the (partial) structure endowed with these endotoxic properties (ref. 9,10). Today, this endotoxically active domain is recognized as lipid A (ref. 1, 2, 9). The structural elucidation of enteric lipid A has been reported (ref. 10, 11) and the chemical synthesis has been completed (ref. 12, 13). The synthetic product, equivalent with E.coli lipid A, had the same biological properties as lipid A obtained from the natural product (ref. 9, 14).
The three designations: lipopolysaccharide, O-antigen and endotoxin are simultaneously used today, depending however, on the specific property or function which should be emphasized (ref. 2, 8).

GENERAL ARCHITECTURE OF LIPOPOLYSACCHARIDES

Distinct architectural elements are shared by lipopolysaccharides from various Gram-negative bacteria, despite of the immense structural diversity existing. The schematic architecture showing the characteristic building blocks observed with lipopolysaccharides from wild-type species is depicted in Fig. 1.
Enterobacterial LPS, but also LPS from species of non-enterobacterial families, is built of three distinct structural regions: region I represents the O-specific chain, region II, the core oligosaccharide and region III, the lipid A moiety (ref. 1, 8, 15). Since each region shows different and highly characteristic constituents, exhibits distinct biological activities and is also under separate genetical control (ref. 16) and has, furthermore, also different taxonomic significance (ref. 17), it is justified and even needed to describe and discuss these three regions separately.

REGION I: THE O-ANTIGENIC REGION

In a number of non-enterobacterial species, e.g. in photosynthetic species (ref. 18), in soil bacteria, e.g. in Thiobacillus versutus (ref. 19), but also in a number of important pathogens, such as Neisseria, Pasteurella, Campylobacter, Bordetella (ref. 20) and in Bacteroides, O-specific chains are completely missing (ref. 8) and their (wild-type) LPS can be depicted as shown in Fig. 1B. In a few species, like in Haemophilus influenzae (ref. 21), and in the phototrophic Rhodocyclus gelatinosus (ref. 22, 23), not only the O-chain (region I), but also the outer core region (region IIa), is completely missing. These LPSs have most reduced polysaccharide chains (Fig. 1C). In general, however, O-specific chains are built up by repeating oligosaccharide units composed of up to seven distinct sugar constituents (ref. 24, 25). Some species have homopolymeric O-chains but this is a rare phenomenon (ref. 26).

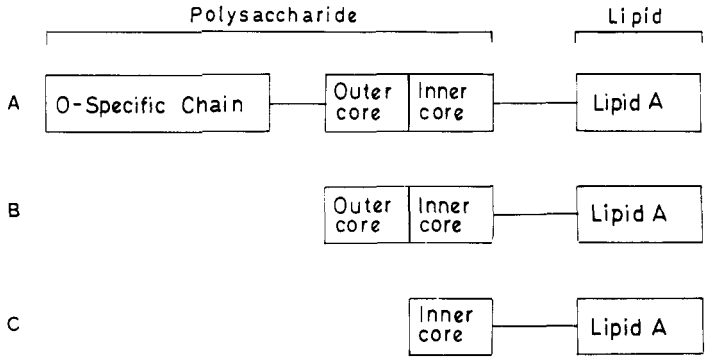


Fig. 1.
General architecture of lipopolysaccharides from (A) enteric bacteria and (B,C) a number of non-enteric bacteria. Modified according to Rietschel et al. (ref. 8).

A few characteristic examples of O-repeating units are shown in Fig. 2 (ref. 24), indicating that neutral and acidic sugars, as well as amino and amphoteric sugars (ref.27, 28), are likewise used as building stones of the individual repeating units. In many cases, e.g. in case of the rare 3,6-dideoxy-hexoses, these unusual components are in terminal position and function as the biologically important "immunodominant" sugars (ref. 1, 25).
Fig. 2 shows the terminal location of paratose (group A of the serological Kauffmann-White scheme) (ref. 7), abequose (group B) and tyvelose (group D_2), which are all linked as branching units to the same linear sugar-chain (ref. 25). In other bacterial genera and families O-methylated, O-acetylated, or otherwise substituted sugars and amino sugars, or even branched sugars (ref. 30, 31), play the role of the immunodominant constituents. In two recent reports (ref. 32, 33), O-specific units with pyruvate in ketal linkage to GlcN, respectively with lysine and alanine linked via their α-amino groups to the carboxylic groups of glucuronic and galacturonic acids (ref. 34) have been described for the swine-pathogenic E.coli O149 and for Proteus mirabilis O27 (ref. 33). The number of newly discovered unusual or extremely rare sugars is still growing. Almost a hundred different sugars or sugar derivatives have so far been encountered in lipopolysaccharides (Table 1) (ref. 35).

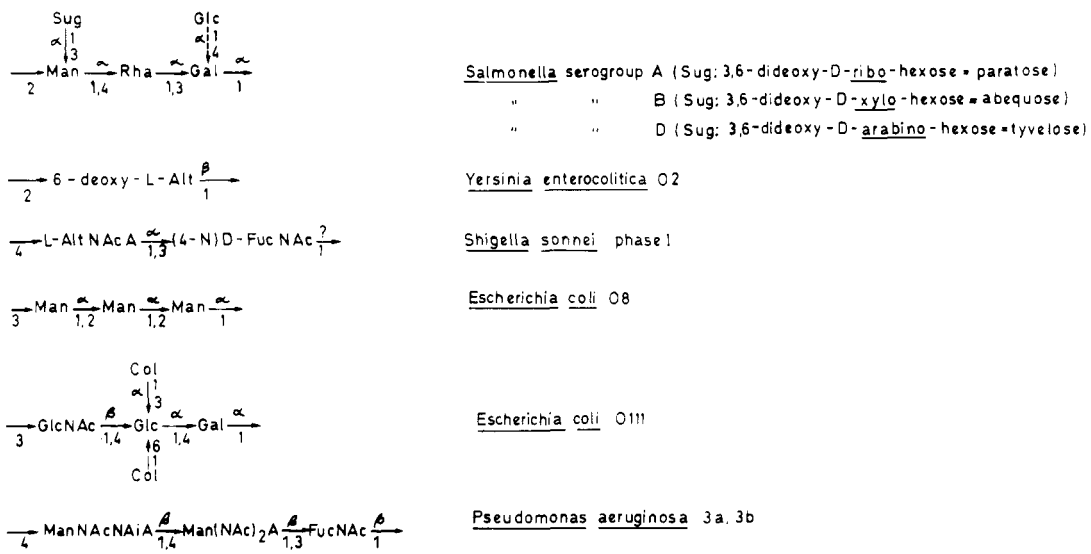


Fig. 2. Examples of different repeating units from lipopolysaccharides of important pathogenic bacterial species. Taken from Kenne and Lindberg (ref. 24).
L-AltNAcA, N-Acetyl-L-altrosaminuronic acid; 4N-D-FucNAc, 2-acetamido-4-amino-2,4-dideoxy-D-fucose; ManNAcNAiA, 2-acetamido-3-acetamidino-2,3-dideoxy-D-mannuronic acid; $Man(NAc)_2A$, 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid; FucNAc, 2-acetamido-2-deoxy-L-fucose.

TABLE I. Sugar and non-sugar constituents identified in lipopolysaccharides. Recently discovered new sugars (ref. 27, 28, and 30) are marked by triangles. Fatty acids are not included. Numbers in parenthesis are numbers of isomers identified. Modified according to Mayer et al. (ref. 35).

Neutral sugars	Amino sugars
Pentose	4-Amino-4-deoxypentose (1)
4-Deoxypentose (1)	2-Amino-2-deoxyhexose (3)
Pentulose	2-Amino-2,6-dideoxyhexose (4)
Hexose (3)	3-Amino-3,6-dideoxyhexose (2)
6-Deoxyhexose (7)	4-Amino-4,6-dideoxyheoxose (3)
3,6-Dideoxyhexose (5)	2,3-Diamino-2,3-dideoxyhexose (1)
Hexulose (1)	2,4-Diamino-2,4,6-trideoxyhexose (2)
6-Deoxyheptose (1)	2-Amino-2-deoxyheptose (1)
Heptulose (1)	
	Acidic sugars
Branched sugars	Hexuronic acid (2)
▲ 3-C-Methyl-6-deoxyhexose (2)	2-Amino-2-deoxyhexuronic acid (3)
▲ 3-C-Hydroxymethylpentose (1)	3-Deoxyoctulosonic acid (1)
▲ 4-C-Hydroxyethyl-3,6-dideoxyhexose (1)	3-O-Lactyl-6-deoxyhexose (2)
	4-O-Lactylhexose (1)
O-Methyl sugars (total 30)	▲ 2,3-Diamino-2,3-dideoxyhexuronic acid (3)
O-Methylpentose	▲ 3-Acetamidino-2-acetamido-2,3-dideoxy-hexuronic acid (2)
O-Methylhexose	
O-Methylheptose	▲ Neuraminic acid (1)
O-Methyl-6-deoxyhexose)	▲ 5,7-Diamino-nonulosonic acid (2)
O-Methyl-2-amino-2-deoxyhexose	
	Non-sugar-constituents
	2-Dihydroxybutanoic acid
	Glycine
	Alanine
	Threonine
	Lysine
	Pyruvic acid
	Ethanolamine

Fatty acids are not included; with modifications according to Mayer et al. (1985). Numbers in parenthesis are the number of isomers identified.

The diversity in O-chain structure and composition may have developed during evolution, at least in endosymbiotic species, in order to escape the host's immune system by again and again developing new specificities on the cell surface and thus to hide the common units, namely lipid A with the inner core region attached to it, which are essential for bacterial growth and multiplication. Thus, the O-chains may protect bacteria against phagocytosis and against the bactericidal action of serum, and they may even allow the bacteria, as found with some intracellular species (e.g. Coxiella burnetii), to survive and to multiply in the otherwise very hostile milieu of phago-lysosomes (ref. 36).
O-specific chains represent, however, also the receptor sites for a number of O-specific (lysogenic) bacteriophages (ref. 37). All lipopolysaccharides, irrespective of their origin, show considerable heterogeneity. In fact, lipopolysaccharide isolates obtained even from a single cultivation, represent a family of macromolecules differing in the length of their individual O-chains (ref. 38, 39). They usually also comprise a fraction having unsubstituted core-stubs (R-LPS). This heterogeneity is due to the way lipopolysaccharide is biosynthesized and it is known that heterogeneity occurs in all three structural regions of LPS (Fig. 1) (ref. 40, 16).

O-chain biosynthesis. Two major routes of O-chain biosynthesis have so far been recognized (ref. 25, 41-43). The importance and the distribution of the two routes for different and phylogenetically distant species have not yet been investigated. The two routes of O-chain biosynthesis are schematically depicted in Fig. 3, and they are designated, following the nomenclature of Shibaev (ref. 43), as the "monomeric" and the "block mechanism". The "block mechanism" has been observed for O-chain biosynthesis in Salmonella typhimurium and in a few related Salmonella serotypes (ref. 25). The first stage of this pathway is the assembly of the oligosaccharide repeating units on the antigen carrier lipid (ACL), which is chemically an undecaprenyl-phosphate (ref. 41). The finished lipid-linked oligosaccharide is then polymerized, still in association with ACL (ref. 41). Following the polymerization, the completed O-chain is transferred to the core-lipid A by the action of the O-translocase system (ref. 16). By this mechanism the chain is growing from the reducing end ("headwards"). Undecaprenyl diphosphate is liberated during the polymerization reaction and its dephosphorylation is necessary before it can again be used for the next turn. Bacitracin was shown to inhibit this dephosphorylation reaction (ref. 25, 43).

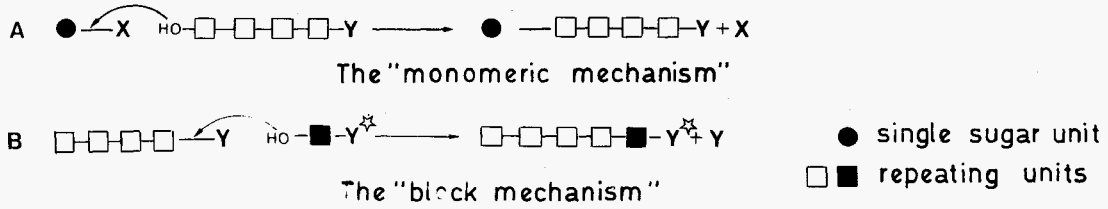


Fig. 3. General reaction mechanisms for the enzymatic polymerization of O-chains, modified according to Robyt (ref. 42): (A) shows the "monomeric mechanism" (according to Shibaev ref. 43), i.e. the addition of an activated monomer (●) to the non-reducing end of a growing chain; (B) shows the "block mechanism", i.e. the addition of an activated repeating unit (■) to the reducing end of a growing O-chain, composed of repeating units (□). X is an activator (nucleotide), Y is a polyprenyl phosphate, usually undecaprenyl phosphate (ACL).

The second mechanism (ref. 25, 43) is dependent on the product of the rfe-gene(s), the function of it, however, is not yet understood (ref. 16). This mechanism seems to be widespread in E.coli and in related enteric bacteria, but has been experimentally proven only for E.coli O8 and O9 (ref. 25, 44). Here, the monosaccharide residues are transferred consecutively from the corresponding glycosyl donors (e.g. from GDP-Man). The acceptor is a monosaccharide residue, which may be part of an oligosaccharide repeating unit, linked to a lipid carrier, integrated into the cell membrane. Here, the polymeric chain grows at the non-reducing end ("tailwards") (ref. 43). How repeating units can be obtained by this mechanism is not yet understood, but methylation analysis (ref. 44) and detergent polyacrylamide gel electrophoresis (PAGE) (ref. 45) clearly demonstrate their existence.

By these pathways a collection of lipopolysaccharide species which differ in the number of their repeating units, and thus in the length of their respective O-chains, are formed. The number of repeating units in the O-chains shows a big fluctuation and may range from 0 (= unsubstituted core stubs) to almost 40 repeating units (ref. 39). This diversity can be electrophoretically visualized with µg-amounts of lipopolysaccharide by detergent-PAGE [either sodium dodecylsulfate (SDS)-PAGE, or deoxycholate (DOC)-PAGE] (ref. 19, 38, 46, 47) (Fig. 4 and 5). The ladder-like banding pattern caused by lipopolysaccharide species with different numbers of repeating units, may be used as a first orientation regarding the heterogeneity (one or more LPS-species present) (ref. 48, 49) or presence and amount of unsubstituted core. It can also be used to recognize the approximate number of sugars in the repeating units [calculated from the observed interspaces of the ladder profile (ref. 45)]. This estimation is, however, only meaningful, when lipopolysaccharide species with a similar or identical lipid A structure are compared (ref. 50), since the binding of detergent to lipid A is influenced by the individual structure of the lipid moiety. This is recognizable from Fig. 4, where lipopolysaccharides having the same lipid A but different number of sugars in the O-repeating unit and those with a different lipid, namely with lipid A_{DAG}, show clearly differing interspaces (ref.50, 23), although the size of the repeating units is similar.

The migration pattern observed with lipopolysaccharide from the fish-pathogen Aeromonas hydrophila is depicted in Fig.5 (ref. 51). Some strains have LPS which shows a regular banding pattern, others show lipopolysaccharides with homologous O-chain-length. The latter possess, in contrast to the former, a

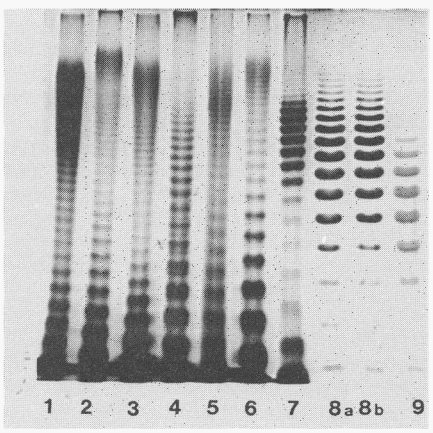


Fig. 4. Deoxycholate-polyacrylamide gel electrophoresis (DOC-PAGE) of lipopolysaccharides from enteric bacteria (with Salmonella type lipid A), lanes 1-7, and from soil bacteria (with lipid A_{DAG}), lanes 8a - 9. Conditions are according to Yokota et al. (ref. 50).

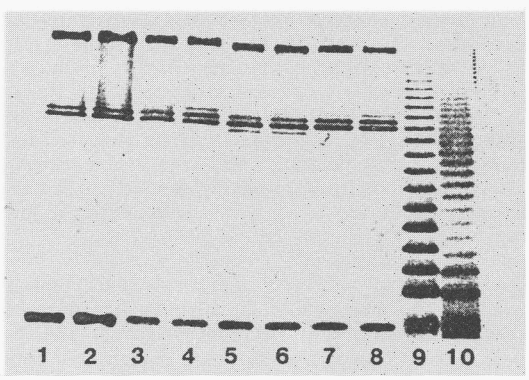


Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Migration pattern of lipopolysaccharides isolated from different strains of the fish-pathogenic Aeromonas hydrophila showing homogenous (lane 1-8) or heterogeneous chain-length O-polysaccharides (lane 9,10). For strains LL1 and TF7 (lanes 3 and 4) the existence of a surface (S-) protein layer was proven [taken from Dooley et al. (ref. 51)].

crystalline protein surface layer, known as S-(surface)-layer (ref. 51, 52). S-layers, frequently encountered in eubacteria and in archaeobacteria (ref. 52) are recognized by electron microscopic methods, especially by freeze-etching techniques (ref. 53). The function of S-layers is not yet understood, but there are indications that they may operate as molecular sieves or as protecting coats (ref. 52) and they may even have an important role as virulence factors for fish-pathogenic Aeromonas species (ref. 51). The production of lipopolysaccharides with homologous O-chain-lengths allows the assembly of the protein lattice on the bacterial surface. With serological techniques it has been shown that O-chains protrude the crystalline surface layer indicating their function as anchors for the S-layers in the outer membrane (ref. 51, 52). Interestingly, an impaired LPS-biosynthesis does not allow the assembly of this protein layer, however, the opposite, namely LPS-biosynthesis, but no protein S-layer, is commonly observed, especially when strains are kept for longer times under laboratory conditions (ref.51, 52). Similar banding profiles with easily recognizable gaps were observed also in DOC-PAGE of a number of phototrophic bacteria, e.g. with LPS from Rhodopseudomonas palustris, Rhodospirillum molischianum and Rhodospirillum rubrum (ref. 23), which assumingly all have protein S-layers as the most distant layer of their cell envelope.

Not much is presently known about the regulation of O-chain polymerization. A mathematical analysis of the distribution pattern of O-chain lengths in Salmonella typhimurium showed convincingly that the synthesis process is regulated and must be length-dependent (ref. 54). The finding, in many LPS-samples, of small amounts of O-methylated sugars (mostly 3-O-methyl-derivatives of D-mannose or L-rhamnose), which occupy exclusively the non-reducing terminal position of O-chains (ref. 55, 56) has been discussed as a signal for cessation of O-chain elongation, at least in those species where the O-chains are built by the "monomeric mechanism", as described above (ref. 43). Examples of lipopolysaccharides with small amounts of terminally linked O-methylated sugars are those from Klebsiella O5, E.coli O8, but also from the cyanobacterium Anacystis nidulans (ref. 56).

REGION II: THE CORE REGION

The finding that Salmonella mutants showing rough (R) colony morphology have defects in their O-chain biosynthesis which results in the synthesis of an R-type LPS with lacking O-chains, had greatly facilitated the elucidation of the structure of the core region, its biosynthesis and its genetic determination (ref. 1, 2, 15). It was recognized that biosynthesis of the core region functions independently from the O-chain biosynthesis and proceeds according to the "monomeric mechanism" discussed above (ref. 16, 57).

It is justified to discuss separately the structure of the outer core region ("hexose-region") and that of the "inner core region" ("heptose-KDO-region"), not only because of their different and characteristic constituents, but also because both regions seem to be differently preserved during bacterial evolution.

The outer core region. The structural diversity of the outer core region is rather limited for enterobacterial lipopolysaccharides. A single core-type is present in all Salmonella serotypes. Five different core-types have been recognized for E.coli serotypes (ref. 58-60) and the same number also for Proteus and related genera (ref. 61, 62) (Table 2) and three for Citrobacter (ref. 63). Fig. 6 shows, as an example, the structures of two pairs of closely related core-types found in E.coli and in Citrobacter (ref. 60, 63). They differ only in the nature (and the linkage) of the branching hexose, depicted in bold letters (Fig. 6). Members of each pair of the core structures exhibit strong serological cross-reactions due to shared epitopes (ref. 58), but they do not cross react with members of the other group.

In non-enterobacterial species the outer core region may also be present, but is also often completely missing. The different core-types can be recognized and distinguished by their characteristic sensitivity pattern towards a set of R-specific bacteriophages (ref. 37, 58).

The inner core region. The KDO-containing inner core region seems to be of lower variability, at least with enterobacterial strains. The make-up of the Salmonella minnesota R7 core region was elucidated using methylation analysis (ref. 64) and is depicted in Fig. 7 (ref. 8, 65). One KDO-residue is present in the main oligosaccharide chain (KDO I), and this is substituted by KDO II (or by a KDO-disaccharide) at position 4. Position 5 of KDO I is further substituted by an α-(1-3)-linked disaccharide of L-glycero-D-manno-heptose, to which the polysaccharide chain is normally attached (ref. 8, 65). Heptose and KDO-residues are usually substituted by charged groups, such as phosphates, pyrophosphates, phosphoryl-ethanolamine or pyrophosphorylethanolamine groups (ref. 66, 67), such leading to an agglomeration of charged residues in this inner part of the core region (ref. 8) and (Fig. 8).

TABLE 2 Different core types of Proteeae, showing common and coretype-specific constituents. according to Kotelko (ref. 62), Basu et al. (ref. 89), and Radziejewska-Lebrecht (unpublished).

Strain		Core type	Common constituents						Core type specific constituents				
			Glc	GalA	KDO	P	EtN	LDHep	Gal	DDHep	GlcN	GalN	4NAra
Proteus mirabilis	028	I	●	●	●	●	●	●	-	-	○	-	○
Proteus mirabilis	1959	II	●	●	●	●	●	●	-	○	○	-	○
Proteus mirabilis	027	III	●	●	●	●	●	●	-	○	-	○	○
Providencia rettgeri	6572	IV	●	●	●	●	●	●	○	-	○	○	-
Morganella morganii	1676	V	●	●	●	●	●	●	○	-	-	○	○

Core type	Structure
E.coli R1	Gal α 1.2 ↓ 1.2 Gal α — Glc ß 1.3 ↓ 1.3 Glc α — Glc 1.3 α — [Hep, KDO, ETN]
E.coli R4	Gal α 1.2 ↓ 1.2 Gal α — Gal ß 1.4 ↓ 1.3 Glc α — Glc
E.coli R3	Glc α 1.2 ↓ 1.2 Glc α — GlcNAc α 1.3 ↓ 1.3 Gal α — Glc
Citrobacter PCM1487	Glc α 1.2 ↓ 1.2 Glc α — GalNAc α 1.3 ↓ 1.3 Gal α — Glc

Fig. 6. The outer core region of two pairs of complete enterobacterial R core-types, which differ solely in the nature (and in the linkage) of the branched sugar unit. [According to Rietschel and Lüderitz (ref. 59) and Romanowska et al. (ref. 63].

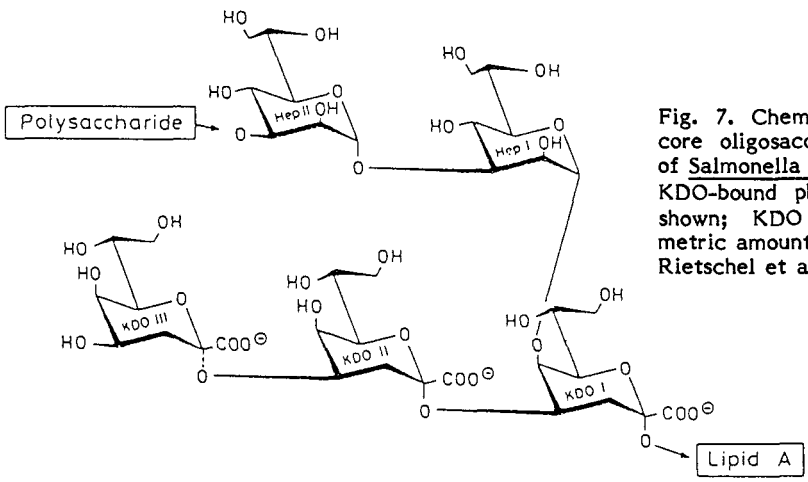


Fig. 7. Chemical structure of the inner core oligosaccharide in lipopolysaccharide of Salmonella minnesota chemotype Rd_1P^-. KDO-bound phosphorylethanolamine is not shown; KDO III is not present in stoichiometric amounts (dotted line). [Taken from Rietschel et al. (ref. 8)].

At least one KDO-residue, or a derivative of it, or, in rare cases, a KDO-analogous substance (Table 3), all having free carboxylic groups at C-1, are found in lipopolysaccharides, indicating their extreme importance for the bacterial cell. The linkage-KDO unit is α-ketosidically bound to the primary hydroxyl group of the non-reducing glucosamine in case of GlcN-containing lipid A's (ref. 8, 72, 73), or to the primary hydroxyl group of a 2,3-diamino-2,3-dideoxy-D-glucose in case of lipid A_{DAG} (ref. 74, 75). The same KDO which carries the polysaccharide chain is functioning also as linkage unit between the polysaccharide chain and the lipid A moiety, (Fig. 7). At least one KDO or KDO-analog is needed for survival and growth of bacteria (ref. 76, 77). In this respect it should be mentioned that biosynthetically lipid A and KDO form a structural unit, since the final acylation of lipid A requires KDO already attached to the lipid A backbone (ref. 78, 79). This seems, however, not to be the case with other bacterial families, such as Pseudomonas, where lipid A acylation is completed, before the attachment of KDO (ref.80). Schindler and Osborn (ref. 81) have shown that the KDO-lipid A-region represents a high affinity combining site for divalent metal ions, such as Ca^{++} and Mg^{++}, and these ions, together with KDO are supposed to be essential for the interlinkage and the assembly of the outer membrane components (ref. 4,5).

Deviating core-structures. In a number of non-enterobacterial LPSs deviating core-structures have been recognized (Fig. 8; ref. 68, 69, 70, 71). So far however, for these core-regions only part-structures have been elucidated. In addition to KDO, the complete R-type and S-form LPSs of Rhodobacter capsulatus contain the structurally related sialic acid in a chain-linked position within the core-region (23). This is surprising, since sialic acid occurs only rarely in microorganisms and has been found so far only in K-antigens.

Thus, phylogenetically distant, non-enterobacterial bacteria appear to have developed differing core-structures during evolution, KDO however is common to all of these core-regions.

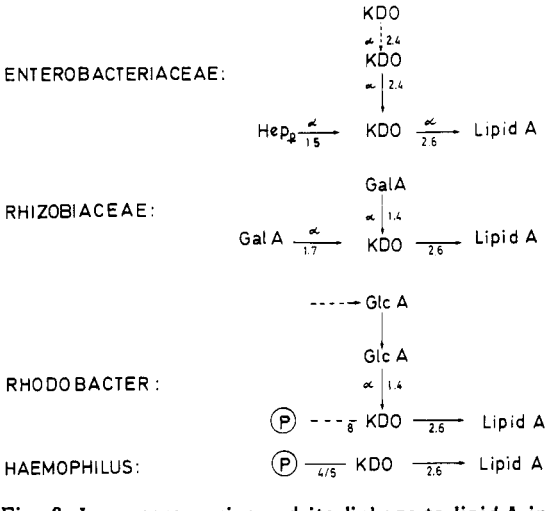


Fig. 8. Inner core region and its linkage to lipid A in some enteric and non-enteric bacteria, showing the agglomeration of negative charges (KDO, phosphate, uronic acids) in this domain. (Data from ref. 65, 68, 69 and 21).

TABLE 3. Occurence of KDO-derivatives and KDO-analoga in LPS

KDOp-=dOclA	Very common
KDOp-5/4-phosphate	Haemophilus influenzae [21] Bordetella pertussis [20]
KDOf	Aeromonas salmonicida [71]
KO=D-glycero-D-talo-octulosonic acid = OclA	Acinetobacter calcoaceticus [67, 70]
2-Keto-3-1,7-dicarboxy-heptonic acid	Acinetobacter calcoaceticus [66]

KDOp/KDOf, pyranosidic and furanosidic form of KDO. Data according to (ref. 20, 21, 66, 67, 70, 71).

KDO – a target for antibiotics

Since KDO is neither present in cells nor tissues from animals or humans, it represents a promising target in the design for new anti-bacterial agents. Inhibitors, which block specifically the incorporation of 3-deoxy-D-manno-octulosonate (KDO), such as 2,8-dideoxy-8-amino-ß-KDO may be used as antibacterial agents. These derivatives and others (ref. 82a, 82b) are potent inhibitors of the enzyme CMP-KDO-synthetase (Fig. 9). Since these molecules are unable to cross the cytoplasmic membrane they showed only little antibacterial activity. If one substitutes, however, the 8-amino-group of the KDO-analog by a dipeptide (e.g. by L-alanyl-L-alanine) the bacterial peptide-permease system transports this prodrug into the cell, where the inhibitor is subsequently released by the action of intracellular peptidases. Good in vitro activity against E.coli and Salmonella was observed with this new antibiotic (ref. 82b).

REGION III: THE LIPID A

The detailed chemical structure of lipid A from enterobacterial and from some non-enterobacterial species has been elucidated recently (ref. 73, 83, 84, 85, 86, 87).
The lipid A moiety of Proteus mirabilis as an example of a highly toxic enterobacterial lipid A (ref. 85), is depicted in Fig. 10. Its lipid A moiety is made up by a 1,4'-bisphosphorylated ß-1,6-D-glucosaminyl-D-glucosamine disaccharide, a structure common to a number of lipid A's isolated from many bacterial genera (ref. 1, 73). The backbone is acylated at positions 2 and 2' with amide-linked 3-hydroxylated fatty acids and at positions 3 and 3' with ester-linked 3-hydroxy fatty acids (in this case with 3-OH-14:0).

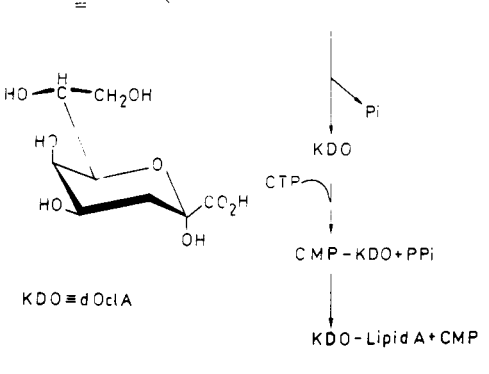


Fig. 9. Structure of KDO, depicted as ketopyranose and the way of its biosynthesis and incorporation as CMP-KDO, modified according to Unger (ref. 78).

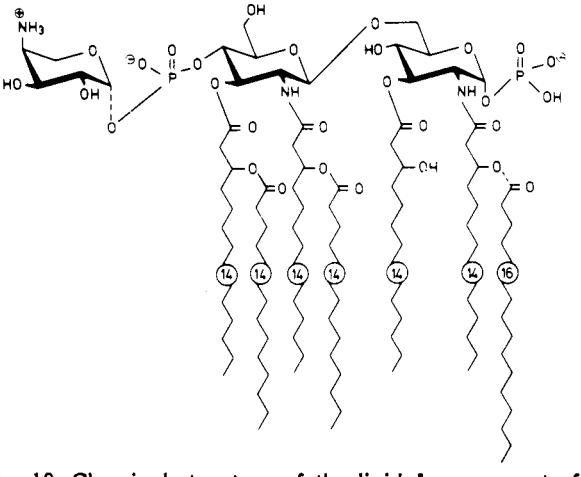


Fig. 10. Chemical structure of the lipid A component of Proteus mirabilis (according to ref. 85). Note the substitution of the ester-linked phosphate by 4-amino-L-arabinopyranose.

As indicated in Fig. 10, the hydroxyl groups of these fatty acids are usually, at least partly, substituted by non-hydroxylated fatty acids, such as myristic acid (14:0) at positions 2' and 3' (on the non-reducing glucosamine) and palmitic acid (16:0) at position 2 (on the reducing glucosamine) (ref. 85). Lipid A of Salmonella and of E.coli shows a very similar distribution of fatty acids (ref. 10), but the latter is lacking one fatty acid residue, resulting in an unsubstituted 3-hydroxylated fatty acid (localized at position 2).

The chemical synthesis of E.coli and Salmonella lipid A has been performed in the group of Shiba and Kusumoto at Osaka (ref. 12, 13) and the biological properties of the natural and the synthetic compounds were shown to be identical in all test systems investigated (9, 88). This was determined for lethality, pyrogenicity, the local Shwartzman activity, changes in the white blood cell count, in activation of macrophages, and in B-cell mitogenicity (ref. 29).

It was, however, noted that the natural products show in most cases a considerable structural heterogeneity, both in the lipid part (non-stoichiometric substitutions of 3-hydroxy fatty acids), but also in the substitution of the phosphate residues by polar head-groups [see as an example the presence of 4-amino-L-arabino-pyranose (Ara-4N in Fig. 10)]. These polar headgroups, such as ethanolamine, phosphorylethanolamine or Ara-4N, do not contribute to the endotoxic properties of lipid A (ref. 29), but may exhibit an indirect influence due to an increase in lipid A (and lipopolysaccharide) solubility. Proteus mirabilis, like the closely related genera Providencia (ref. 89) and Morganella, shows in contrast to E.coli and other enterobacterial species, an almost complete substitution of the ester-linked phosphate by Ara-4N (ref. 85, 62). This substitution was proposed by Vaara et al. (ref. 90) to be the structural reason for the observed resistence of these species and genera towards the antibiotic polymyxin. This assumption could recently be confirmed by the isolation and characterization of an Ara-4N-lacking mutant (R4/O28) from Proteus mirabilis O28 (ref. 91). This mutant proved to be as sensitive towards polymyxins B and E_2 as E.coli and Salmonella strains tested in parallel, quite in contrast to the polymyxin-resistent wild-type strain Proteus mirabilis O28 (ref. 92).

The polycationic polymyxins apparently need the unsubstituted ester-linked phosphate of the lipid A backbone to attach to the bacterial surface (ref. 93) as a first event in their antibiotic action.

The multiform biological and pathophysiological properties of endotoxins and their synthetical equivalents have been extensively described and covered in recent reviews (ref. 8, 29) and will therefore not be discussed here in extenso.

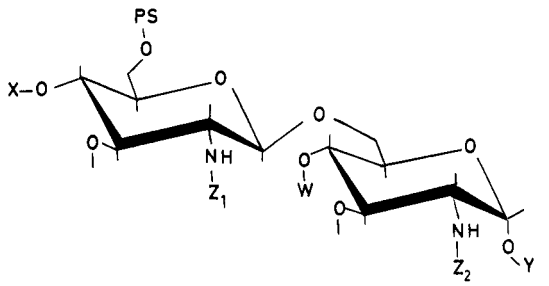
LIPID A VARIANTS OF LOW TOXICITY

Non-toxic or weakly toxic lipopolysaccharides are rather widespread in bacteria, phylogenetically not closely related or distant to Enterobacteriaceae, such as in phototrophic bacteria (ref. 18, 74, 94), in nodulating bacteria (Bradyrhizobium species) (ref. 95), or in soil bacteria (Nitrobacter and Thiobacillus species) (ref. 50). They show structurally deviating lipid A moieties, designated as lipid A variants, which differ from the toxic lipid A either by their lipid A sugar backbone or by their fatty acid spectrum (ref. 86). These lipid A variants can be grouped into two major clusters (ref. 19). The first group has the same amino sugar disaccharide, namely the ß-1,6-linked D-glucosaminyl-D-glucosamine-disaccharide, as found with the toxic enterobacterial lipid A (Fig. 11), some species lack, however, the phosphate groups (ref. 87), others have the amide-linked 3-hydroxy-fatty acids either partly (Rhodobacter sphaeroides) or completely (Rhodobacter capsulatus 37b4) replaced by 3-oxo-myristic acid (3-oxo-14:0) (ref. 96, 97). In other lipid A's, the otherwise free hydroxyl group at position 4, is substituted by an additional (non-acylated) glucosamine residue, as in the case of Rhodocyclus tenuis (ref. 35, 86). All these lipid A variants exhibit low or even lacked lethality and pyrogenicity (ref. 94). Of special interest is the serologically, with Salmonella lipid A completely cross-reacting lipid A-variant present in Rhodobacter capsulatus 37b4 (ref. 97), which shows a toxicity which is 10^3-10^4 times lower than the one of Salmonella lipid A, assayed in the same system, namely the galactosamine-treated mouse (ref. 98) (Table 4).

TABLE 4. Biological properties of lipopolysaccharides with variants of lipid A or lipid A_{DAG}. (According to Galanos et al. (ref. 94) and Lohmann-Matthes et al. (unpublished).

Source of LPS	LIPID A charact. [HexN	FA-A	P]	Lethality [x 10^{-2}µg/kg]	Pyrogenicity [x 10^{-3}µg/kg]	TNF-Induction [Cr^{51} release]
Salmonella	GlcN	3-OH	+	1	1	+++
Rc. gelatinosus	GlcN	3-OH	+	1	1	+++
Rb. sphaeroides	GlcN	3-OH 3-Oxo	+	10^3 - 10^4	10^3	+
Rb. capsulatus	GlcN	3-Oxo	+	10^3-10^4	ND	+
Rp. viridis	DAG	3-OH	-	10^2 - 10^3	10^4	++/+++

HexN, hexosamine; DAG, 2,3-diamino-2,3-dideoxy-D-glucose; FA-A, amide-linked 3-hydroxyl or 3-oxo-fatty acid. Lethality, LD_{50} in adrenalectomized (ref. 94) or galactosamine treated mice (ref. 98); Pyrogenicity, MPD-3 values (ref. 94) in rabbits; TNF, tumor necrosis factor induction, determined as ^{51}Cr-release from TNF-sensitive L 929 transformed fibroblast target cells.



Species	W	X	Y	Z_1	Z_2
Rhodocyclus tenuis					
2761	GlcN	Ara4N-P	Araf-P	3-O(16:0)-10:0	3-OH-10:0
Rhodobacter capsulatus					
37b4	H	ETN-P	ETN-P(P)	3-oxo-14:0	3-oxo-14:0
Rhodobacter sphaeroides					
ATCC17023	H	P	P	3-O(Δ^7-14:1)14:0	3-oxo-14:0
Rhodomicrobium vannielii	H				
ATCC17100	H	Man/Ac	H	?	3-OH-16:0

Fig. 11 Structural make-up of lipid A variants of low or lacking toxicity from phototrophic bacteria sharing the ß-1,6-glucosamine disaccharide as sugar backbone. According to Mayer et al. (ref. 19). Ara4N-P, 4-amino-L-arabino-pyranose-1-phosphate; Araf-P, D-arabino-furanose-1-phoshate; ETN-P, ethanolamine-phosphate, ETN-PP, ethanol-amine-pyrophosphate; Man, D-mannopyranose; Ac, O-acetyl-residues.

The second main group of lipid A variants has the rare 2,3-diamino-2,3-dideoxy-D-glucose as lipid A backbone sugar, first reported from lipid A of Rhodopseudomonas viridis (ref. 99) and otherwise not found in any other natural product so far. The backbone of lipid A with diaminoglucose as backbone sugar, recently designated as lipid A_{DAG} (ref. 74), may occur in a monosaccharidic form, as found in Rhodopseudomonas viridis (Fig. 12) and in Phenylobacterium immobile (ref. 75), but it can also occur in a disaccharidic form, as recently reported for lipid A_{DAG} from Pseudomonas diminuta (ref. 100). The latter has been reported to have notable pyrogenicity and lethality (ref. 100), but the former (R.viridis Lipid A) is non-toxic and non-pyrogenic (ref. 94).
It is of interest that lipid A synthase of E.coli, accepts not only UDP-activated N,O-2,3-diacyl-D-glucosamine (UDP-lipid X) to form the tetra-acyl-glucosamine-disaccharide-1-phosphate (ref. 79) but also the 2,3-diamino-derivative of lipid X, namely lipid X_{DAG} (ref. 74,101). Thus it was possible to obtain in vitro backbone structures with "mixed disaccharides" with either ß-1,6-diaminoglucosyl-glucosamine-1-phosphate or with ß-1,6-glucosaminyl-diaminoglucose as sugar backbone, depending whether UDP-lipid X and lipid X_{DAG} or UDP-lipid X_{DAG} and lipid X were present in the incubation mixture (ref. 74, and F. Unger, Vienna, personal communication).

So far, 2,3-diamino-2,3-dideoxy-D-glucose has been detected in lipopolysaccharides from about 25 species belonging to twelve different genera (ref. 19, 74), showing that lipid A_{DAG} is rather widespread amongst non-enterobacterial species. It has been found also in important pathogens, like in Brucella abortus and Brucella melitensis (ref. 102). There is strong indication that "mixed" lipid A backbones do also exist in phototrophic bacteria, especially in Chromatiaceae although they have so far not been definitely proven (ref. 74).
For a significant number of species with lipid A variants as part of their lipopolysaccharides it was demonstrated that their distinct composition matches excellently with their phylogenetical position

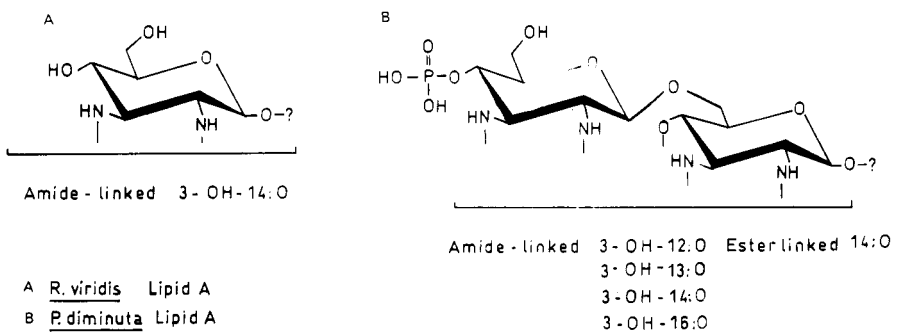


Fig. 12. Monosaccharidic (A) and disaccharidic (B) lipid A_{DAG} isolated from Rhodopseudomonas viridis and Pseudomonas diminuta lipopolysaccharides. Modified according to Weckesser and Mayer (ref. 74).

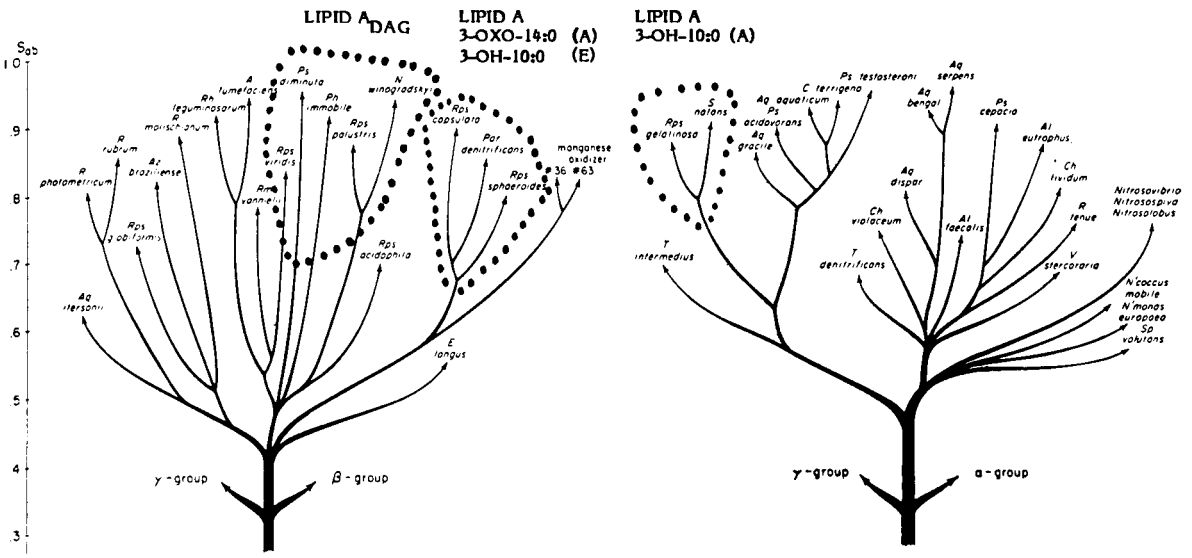


Fig. 13. Correspondence of distinct lipid A types or variants and 16 S rRNA catalogues within the α (left) and the ß (right) subgroups of purple and related non-phototrophic bacteria. Subgroups according to Woese et al. (ref. 104 and 105); lipid A-types according to Weckesser and Mayer (ref. 74) and Masoud (unpublished results).

based on 16S rRNA homologies (ref. 103, 74). As an example, the α-and the ß-branch of the phylogenetical tree of purple bacteria are shown in Fig. 13, which also shows the three distinct lipid A variants recognized so far (ref. 104, 105, 106 and unpublished data).
Non-toxic lipopolysaccharides might turn up to be medically useful in competing with toxic ones, in blocking possible receptor sites, e.g. on macrophages or on other target cells, and/or leading to endotoxin tolerance phenomena (ref. 8). The biological properties of non-toxic lipid A's are presently investigated by several groups as to their pharmacological possibilities.

CONCLUDING REMARKS

It has been shown that O-chains of lipopolysaccharides exhibit a very high structural variability. The more proximal (to lipid A) region of the outer core still shows considerable structural variation, but the inner core region and lipid A express only limited variability and this variability is of taxonomic and even of phylogenetic significance (ref. 17, 74). One can conclude from these findings that those partial structures which are essential for bacterial growth and multiplication are forming the non-variant domain of lipopolysaccharides (ref. 8). KDO or distinct KDO-analogs (Table 2) are needed for bacterial survival. It can be imagined that lipid A, and likewise lipid A variants, are not more than highly elaborated and easily modifiable lipid anchors for KDO residues, which may keep KDO in a correct position on the bacterial surface. It is known that KDO in co-operation with divalent cations, such as Ca^{++} and Mg^{++} plays an essential role in the organization and in the inter-linkages of the components of the outer membrane (ref. 81, 4, 5). From the fact that lipid A of the phototrophic water bacterium Rhodocyclus gelatinosus is of very high lethal toxicity (ref. 94), one recognizes, that the endotoxic principle was invented early in evolution (ref. 2) and independent from a specific host-parasite interaction. Endotoxicity could just be an accidental property of a molecule used as an anchor of KDO in the outer membrane (ref. 19). The diversity in O-chain structures developed especially in endosymbiotic species to escape the immune system of the host and to hide the common and essential KDO-lipid A region from the attack of the immune system, has already been mentioned.

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