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Chemical Structure of the Core Region of Lipopolysaccharides — an Update

リポ多糖のコア領域の化学構造 ー 最新版

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Abstract

Lipopolysaccharides (LPS) are the endotoxins of Gram-negative bacteria and very well known for their immunological, pharmacological and pathophysiological effects displayed in eucaryotic cells and organisms. To date, much emphasis has been put on the elucidation of the chemical structures of LPS and on their relation, or that of substructures, to the various biological effects. The lipid part of LPS, the lipid A, was proven to represent the toxic principle of endotoxin. However, lipid A toxicity depends strongly on its structure, and is influenced by a second region of LPS, the core region, that is covalently linked to lipid A. Also, the core region possesses immunogenic properties. Therefore, complete structural analyses of the core region and the comparison of its structures with biological features of LPS are of high importance for a better understanding of LPS action, and one prerequesite for strategies aimed at the treatment of endotoxicosis. In the past, quite a number of structures of the core regions from various Gram-negative bacteria were published and summarized in several overviews. The present review adds to this knowledge those structures that were published between October 1998 and December 2001.

要　約

リポ多糖 (LPS) はグラム陰性細菌の内毒素であり、真核生物の細胞や組織に対して免疫学的、薬理学的、病態生理学的な作用を示すことが知られている。現在までに、LPS や類縁体、その部分構造について、様々な生物活性に対応する化学構造解析が精力的に行われてきている。LPS の脂質部分であるリピドAは内毒素の活性中心であることが証明されている。しかし、リピドAの毒力はそれ自身の構造に強く依存するだけでなく、リピドAに共有結合した LPS の一部分であるコア領域の影響も受けている。コア領域もまた免疫学的作用を示す。すなわち、LPS の作用を理解し内毒素症の治療に結びつけるためには、コア領域の構造を解析しその構造と活性とを比較することが重要である。これまでに、さまざまなグラム陰性細菌のコア領域の構造が発表されており、それぞれ総説にまとめられている。この論文では、1998 年 10 月から 2001年 12 月までに発表された構造について概説した。

A. Introduction

Lipopolysaccharides (1) can be classified into two types which are characterized by the size of the saccharide portion, i.e. smooth- and rough-form (S- and R-form) (2–4). Both types consist of lipid A and, covalently linked to it, a saccharide portion composed of up to fifteen sugars, the core region (4–6). In S-Form LPS, this core region is substituted by the O-specific polysaccharide (7, 8). Both LPS types are present in wild-type Gram-negative bacteria, S-form for example in *Escherichia coli* or *Vibrio cholerae*, and R-form in *Neisseria meningitidis* or *Bordetella pertussis*. Since mutants that are not able to synthesize a minimal core structure are not viable, the core region and lipid A represent a common structural unit occurring in all LPS and important for viability and membrane function of Gram-negative bacteria. Herein, the chemical structure of the core region of LPS is reviewed. Since part of the work was summa-

A. 序　論

リポ多糖 (1) は、その糖鎖部分の長さに応じてＳ (Smooth) 型とＲ (Rough) 型の２種類に分類することができる (2-4)。双方とも、リピドAおよびそこに共有結合しているコア領域と呼ばれる15残基までの糖鎖を含んでいる (4-6)。Ｓ型 LPS では、このコア領域にＯ抗原多糖がさらに結合している (7､8)。それぞれの型の LPS は、Ｓ型であれば *Escherichia coli* (大腸菌) や *Vibrio cholerae* (コレラ菌)、Ｒ型であれば *Neisseria meningitidis* (髄膜炎菌) や *Bordetella Pertussis* (百日咳菌)といった野生型のグラム陰性細菌に存在する。コア領域を生合成できない変異株は生存できないことから、コア領域とリピドAはすべての LPS に存在する共通構造であり、グラム陰性細菌の生存と膜の機能性にとって重要であるといえる。ここでは、LPS のコア領域の化学構造

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rized recently (3–6), this review comprises only the structures that have been published from October 1998 through December 2001.

B. General Structural Features of the Core Region

When the structures of core regions from different bacterial origin are compared, it appears obvious that the chemical variation in this part is more limited than in the structures of O-specific polysaccharides. Still, there is only one structural element which is present in **all** core regions, i.e. the 3-deoxy-D-*manno*-oct-2-ulopyranosonic acid [Kdo, (9)] residue that links the core region to the lipid A. A majority of core regions possesses in addition L-*glycero*-D-*manno*-heptopyranose (L,D-Hep) and the oligosaccharide L-α-D-Hep-(1→3)-L-α-D-Hep-(1→5)-[α-Kdo-(2→4)]-α-Kdo. In this review, for the residues of this structure the abbreviations Hep II, Hep I, Kdo II, and Kdo I, respectively, are used. Structural variations in these core regions occur by different substitutions of this oligosaccharide by other sugar substituents and, in most of the investigated core regions, also by phosphate residues. In addition to L,D-Hep, few LPS contain D-*glycero*-D-*manno*-heptopyranose (D,D-Hep) which was identified as the biosynthetic precursor of L,D-Hep (10-14). However, there are also some LPS that contain only D,D-Hep (15) or even lack heptose, *e.g.* in *Acinetobacter* (16–20) or *Chlamydia* (21). D,D-Hep may be condensed to a heptoglycan which was identified in strains of *Klebsiella pneumoniae* (α-(1→2)-linked) and *Helicobacter pylori* (α-(1→3)-linked). Either Kdo I [in *Acinetobacter* (16–18)] or Kdo II [in *Burkholderia cepacia* (22, 23)] may be replaced by another, stereochemically similar sugar, *i.e.* D-*glycero*-D-*talo*-oct-2-ulopyranosonic acid [Ko (24)]. Its biosynthesis and the regulation of the exchange between Kdo and Ko have not been elucidated so far.

During the past 20 years, several methodological problems regarding the structural analysis of the core region have been solved. First, the difficult chemistry of Kdo could be overcome in the mid 1980s (25), providing methods that elucidated the structure of the Kdo region of the core. The second problem was the analysis of the phosphate substitution of core sugars, a problem which has still not been fully overcome. We have developed a degradation pathway to obtain phosphorylated oligosaccharides from LPS by using successive mild hydrazinolysis and strong alkali treatment and isolation of pure compounds by high-performance anion-exchange chromatography (HPAEC) (26). The application of this methodology has led to the characterisation of various core regions from bacteria of remote genetic origin. Still, phosphodiester, diphosphate, diphosphodiester, acetyl, and carbamoyl groups which are present in many LPS are cleaved under strong alkaline conditions and their positions can thus not be identified. Also, the alkaline treatment gives rise to phosphate migration in case of substitution by 2-aminoethyl phosphate [PE (27–29)] and to β-elimination of 4-

について概説する。この論文では 1998 年 10 月から 2001年 12 月に発表された構造に絞って概説しているので、それ以前のものについては総説を参照されたい (3-6)。

B. コア領域の一般構造

異なる細菌由来のコア領域の構造を比較すると、O抗原多糖の構造ほどには化学的な多様性が存在しないことがわかる。それでも、すべてのコア領域に存在する構成成分は、コア領域とリピドAをつなぐ Kdo (3-deoxy-D-*manno*-oct-2-ulopyranosonic acid) (9) ただ一つである。多くのコア領域は、ヘプトースの一種である L,D-Hep (L-*glycero*-D-*manno*-heptopyranose) を含む L-α-D-Hep-(1→3)-L-α-D-Hep-(1→5)-[α-Kdo-(2→4)]-α-Kdo 構造のオリゴ糖を持っている。この総説では、この構造の残基の略称として Hep II、Hep I、Kdo II、Kdo I を使用する。コア領域の構造多様性は、このオリゴ糖に他の糖残基やリン酸基といった異なる置換基が結合することで生じている。ある種の LPS は、L,D-Hep に加えて L,D-Hep の生合成前駆体である D,D-Hep (D-*glycero*-D-*manno*-heptopyranose) を含んでいる (10-14)。しかし、LPS の中には、D,D-Hep だけ含んでいたり (15)、*Acinetobacter* (アシネトバクター)(16-20) や *Chlamydia* (クラミジア)(21)のようにヘプトースを含んでいないものもある。D,D-Hep は、*Klebsiella pneumoniae* (肺炎桿菌) の α-(1→2) 結合ヘプトグリカンや *Helicobacter pylori* (ピロリ菌) の α-(1→3) 結合ヘプトグリカンのように多糖として濃縮されているのかもしれない。*Acinetobacter* の Kdo I (16-18) や *Burkholderia cepacia* の Kdo II (22、23) などは、同様の立体化学を持つ Ko (D-*glycero*-D-*taro*-oct-2-ulopyranosonic acid) (24) に置き換わっているかもしれない。その生合成と Kdo と Ko の交換の制御については、これまでのところ明らかにされていない。

コア領域の構造解析に関する手法的な問題のいくつかは、この 20 年間に解決されてきた。まず、Kdo の化学的な扱いにくさが 1980 年代の中頃に克服され (25)、コアの Kdo の構造解析が可能になった。2 つ目はコア糖残基のリン酸置換基の問題であるが、これは完全には解決されていない。我々は、LPS をヒドラジン分解後に強アルカリで処理することによって分解し、さらに高性能陰イオン交換クロマトグラフ (HPAEC; high-performance anion-exchange chromatography) でリン酸化オリゴ糖を単離するという経路を開発した。この手法は、遺伝的にかけ離れた様々な細菌由来のコア領域の解析に応用されている。それにもかかわらず、多くの LPS に存在するリン酸ジエステルや二リン酸、二リン酸ジエステル、アセチル、カルバモイルといった置換基は強アルカリ処理時に切断されてしまうため、それらの結合位置を同定することはできない。またアルカリ処理は、2-アミノエチルリン酸 (PE) 置換の場合のリン酸の転移 (27-29) や、4 位置換ガラクツロンサン (GalA) の β 脱離 (30, 31) も

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substituted galacturonic acid (GalA) residues (30, 31). However, the combination of the data obtained from these analyses with those obtained from the analyses of core oligosaccharides isolated after hydrolysis of LPS in e.g. 1% aqueous acetic acid overcomes the problem and leads to complete structures, as shown *e.g.* in (32). Recently, combined capillary electrophoresis-electrospray mass spectrometry was shown to be of high value in the characterization of different LPS glycoforms (33).

If not mentioned otherwise in the following presentation, sugars are present as α-D-pyranosides.

C. Core Structures

C-1. Heptose-Containing Core Regions

C-1-1. *Enterobacteriaceae* (Table I)

C-1-1-1. The *Salmonella enterica* Type Core Oligosaccharides

S. enterica type core structures contain as common structural element L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo which is substituted at O-3 of Hep II by Glc. Heptose residues are phosphorylated and position O-4 of Hep I is not substituted by a saccharide.

C-1-1-1-1. *Escherichia coli*

Recently, the linkage points of the phosphate groups in the core type R3 and the structure of the inner core region of R4 were characterized (34). The latter structure is very similar to the inner core structures of the core types R-2 and K-12 (Table I); no substitution of the branching Kdo occurs.

C-1-1-1-2. *Hafnia alvei*

In addition to the single carbohydrate backbone that had been identified in the core region of several strains of *H. alvei*, three other core types have been elucidated (35–37), the structures of which are shown in Table 1. Surprisingly, the core region of LPS from strain 39 has the structure of the R1S core of *S. enterica* (6) and that of LPS from strains 23 and 1222 possesses the structure to the *E. coli* R4 type (compare Table I). The core structure of LPS from strains 1185 and 1204 is very similar to that published earlier, with the single difference that a Gal-(1→3)-Glc disaccharide substitutes O-3 of Hep II.

C-1-1-2. Core Oligosaccharides Different to the *Salmonella enterica* Type

Here the common partial structure L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo is not substituted at O-3 of Hep II by Glc and heptose residues are not generally phosphorylated. Position O-4 of Hep I is substituted by a hexose residue or oligosaccharide.

C-1-1-2-1. *Klebsiella pneumoniae*

As a third core region of LPS from *K. pneumoniae*, that of S-form LPS of serotype O3 was investigated (38). Part of the structure is identical to the structures published for R-form LPS from serotypes O1 (39) and O8 (40). Thus, Hep I carries at

引き起こす。しかし、これらの分析データと、１％酢酸水溶液等による LPS の加水分解生成物から得たコアオリゴ糖の分析データを組み合わせることで問題を克服し、文献 (32) のような完全構造を導くことができる。最近は、キャピラリー電気泳動とエレクトロスプレー質量分析法を組み合わせることによって、LPS の糖構造の差異を決定できることも示されている (33)。

以下の項では、特に記載のない限り糖は α-D-ピラノース構造を示している。

C. コアの構造

C-1. ヘプトースを含んだコア領域

C-1-1. *Enterobacteriaceae*（腸内細菌科）(Table I)

C-1-1-1. *Salmonella enterica*（サルモネラ菌）型コアオリゴ糖

S. enterica 型のコアの構造は、共通部分として L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo の Hep II のO-3位に Glc が結合したものを含んでいる。ヘプトース残基はリン酸化されており、Hep I のO-4位は糖置換を受けていない。

C-1-1-1-1. *Escherichia coli*（大腸菌）

最近、R3型コアのリン酸基の結合位置と、R4型の内部コア領域の構造が決定された (34)。後者の構造はR2型とK12型の内部コア構造に非常に類似しており (Table I)、側鎖 Kdo の置換は見られなかった。

C-1-1-1-2 *Hafnia alvei*

これまでは *H. alvei* のコア領域として一種類の糖骨格しか同定されていなかったが、さらに Table I に示した構造を持つ３つのコアが明らかになった (35-37)。特に、strain 39 由来 LPS のコア領域は *S. enterica* のR1S型コアの構造であり、strain 23 と strain 1222 由来のコアが *E. coli* のR4型の構造であることは注目に値する。strain 1185 と strain 1204 は、Hep II のO-3位に Gal-(1→3)-Glc が置換していることを除いて、以前に発表されたものと類似している。

C-1-1-2. *Salmonella enterica* 型と異なるコアオリゴ糖

ここではL,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo の Hep II のO-3位に Glc が結合しておらず、またヘプトースがリン酸化されていないものを扱う。Hep I のO-4位はヘキソースやオリゴ糖で置換されている。

C-1-1-2-1. *Klebsiella pneumoniae*（肺炎球菌）

K. pneumoniae の三番目のコア領域として、serotype O3のＳ型 LPS が解析された (38)。構造の一部は、serotype O1 (39) や O8 (40) 由来のＲ型 LPS のものと一致している。Hep I のO-4位は β-GalA-(1→6)- β-Glc で、Hep II のO-3位は GalA （serotype O1

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Table I. The structures of particular core regions of enterobacterial LPS. Where not stated otherwise, sugars are α-D-pyranosides.

Escherichia coli		*Ref.*
Core type	Structure	
R3	$P^{a,b}$ ↓ 4 P^{b} ↓ 4 Glc-(1→2)-Glc-(1→2)-Gal-(1→3)-Glc-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[c] 3 ↑ 1 GlcNAc[d] 7 ↑ 1 L,D-Hep 7 ↑ 1 GlcNAc[d] 4 ↑ 2 Kdo[d]-(2→4)-Kdo Strain J-5	(34)
R4	P^{b} ↓ 4 P^{b} ↓ 4 Gal-(1→2)-Gal-(1→2)-Glc-(1→3)-Glc-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[c] 4 ↑ 1 Gal 7 ↑ 1 L,D-Hep 4 ↑ 2 Kdo	(34)

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Hafnia alvei Strains 1185 and 1204	P ↓ 4 PPE ↓ 4 Gal-(1→3)-Glc-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo 7 ↑ 1 L,D-Hep	(36)
Strains 23 and 1222	*E. coli* R4 core (see above)	(35)
Strain 39	*S. enterica* R1S core	(35, 6)

K. pneumoniae[x]

Serotype	Structure	
O3	L,D-Hep[a]-(1→4)-Kdo-(2→6)-GlcN-(1→4)-GalA-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[c] 7 ↑ 1 L,D-Hep; 4 ↑ 1 β-Glc; 4 ↑ 2 Kdo 7 ↑ 1 β-GalA; 6 ↑ 1 β-GalA[a]	(38)

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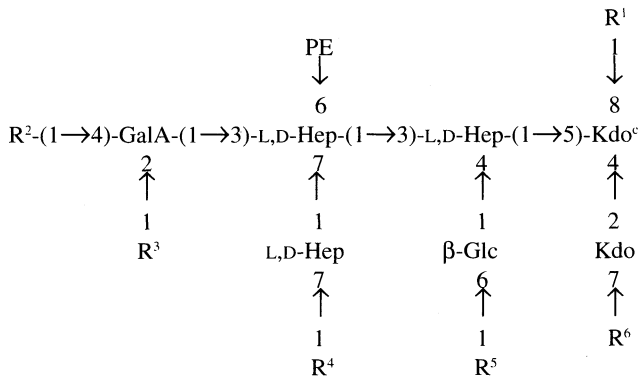
Proteus General structure



Serotype/ strain	R^1	R^2	R^3	R^4	R^5	R^6	*Ref.*
mirabilis O27	H	β-Glc-(1→5)-(1*S*)-Gal*o*NAc[e]-(1→4,6)-GalN	β-Gal[a]-(1→7)-D,D-Hep	β-GalA[a]-(1→7)	GalA[a]-(1→6)	P[a]	(43)
vulgaris OX2, O4, O8 *penneri* 2,11,17, 19, 107	β-L-Ara4N[a,f]	β-Gal[a]-(1→4)-(1*S*)-Gal*o*NAc-(1→4,6)-GalN	L,D-Hep-(1→2)-D,D-Hep	H	H	H	(44, 47)
vulgaris O25	β-L-Ara4N	β-GlcNAc-(1→2)-β-Gal-(1→6)-GlcN **or** β-Kdo-(2→3)-β-Gal-(1→6)-GlcN	L,D-Hep-(1→2)-D,D-Hep	H	H	H	(45)
mirabilis O28	β-L-Ara4N[a]	β-GalALys[g,h]-(1→3)-β-GlcNAc-(1→6)-GlcNGly[i]	H	β-GalA[a]-(1→7)	H	H	(46)

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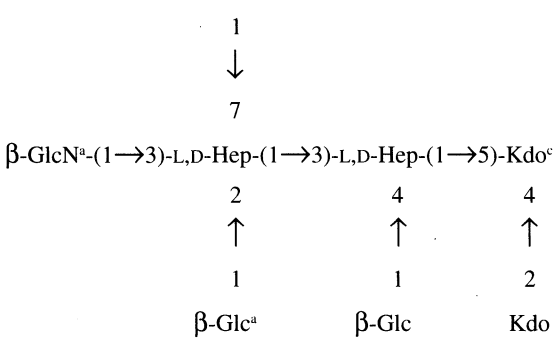
penneri 40	β-L-Ara4N[a]	Glc-(1→6)-β-Gal-(1→4)-(1*S*)-Gal*o*NAc-(1→4,6)-GalN	L,D-Hep-(1→2)-D,D-Hep	H	H	H	(47)
mirabilis O3/S1959 and R110/1959	β-L-Ara4N[a]	D,D-Hep-(1→6)-GlcN	D,D-Hep[a]	β-GalA-(1→7)	H	H	(48)
mirabilis O6	β-L-Ara4N[a]	β-Qui4NAlaAla[k]-(1→3)-Gal-(1→6)-GlcNAc-(1→4)-GalN	D,D-Hep	β-GalA[a,l]-(1→7)	H	H	(49)
mirabilis O48	β-L-Ara4N[a]	Glc-(1→4)-[β-Gal-(1→6)-]-GalN	D,D-Hep	β-GalA[a,l] -(1→7)	H	H	(49)
mirabilis O57	β-L-Ara4N[a]	β-Qui4NAlaAla-(1→3)-GalNAc-(1→6)-Glc-(1→4)-GalN	D,D-Hep	β-GalA[a,l] -(1→7)	H	H	(49)

Yersinia enterocolitica

Serotype/strain O:3/8081-c-R2 (51)



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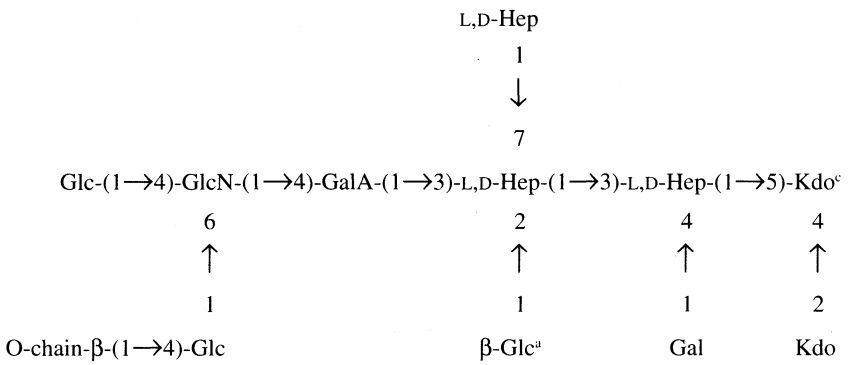
Plesiomonas shigelloides (52)



Unless stated otherwise, sugars are α-D-pyranosides. Kdo III is present in non-stoichiometric amounts. [a] Non-stoichiometric substitution. [b] Product obtained after de-*O*-acylation. Further substituents are not known. [c] This residue was identified to link the core region to GlcN II of lipid A. [d] Partially *N*-acetylated in the complete core. [e] Gal*o*NAc, open form of GalNAc, in acetal linkage. [f] Ara4N, 4-amino-4-deoxy-arabinopyranose. [g] GalALys, GalA amidated with lysine. [h] PE is present at O-4 in about 50% of this residue. [i] GlcNGly, GlcN amidated with glycine. [k] Qui4NAlaAla, 4-*N*-(L-alanyl-L-alanyl)-4-amino-4,6-dideoxy-glucopyranose. [l] This residue is partially amidated with either putrescine or spermidine.

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O-4 the disaccharide β-GalA-(1→6)-β-Glc, and Hep II is substituted at O-3 by GalA which is in turn substituted at O-4 by GlcN (as in serotype O1). However and in difference, Hep III is in the core region of serotype O3 LPS substituted at O-7 by β-GalA. The most striking structural feature is the presence of the disaccharide L,D-Hep-(1→4)-Kdo that substitutes O-6 of the GlcN residue. This trisaccharide was also found in other S-form LPS from serogroups O1, O2, O4, O5, O8, and O12 (38). In difference to the core structure of a rough type LPS from serogroup O1 (which had not been investigated with regard to the presence of the trisaccharide L,D-Hep-(1→4)-Kdo-(2→6)-GlcN) (39), no D,D-heptoglycan was identified in the core region of S-form O1 LPS. In the rough type LPS this heptoglycan substitutes the GlcN residue at the same position (O-6) as the disaccharide L,D-Hep-(1→4)-Kdo does in the S-form LPS. Thus, a substitution at this position seems to be crucial for both, S- and R-form LPS of *K. pneumoniae* O1.

C-1-1-2-2. *Proteus mirabilis*

A highly significant work has been done on the core regions of LPS from various serotypes of the genus *Proteus* (41–49). The structures are shown in Table I. All core structures possess a common carbohydrate backbone which is L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-[Kdo-(2→4)-]Kdo. Hep I is substituted at O-4 by β-Glc, and Hep II carries GalA at O-3 and PE at O-6. This backbone is differently substitued by various sugars which determine the structural specifities of the core regions.

There are two striking features in the cores of *Proteus* LPS: for the first time, an open-chain acetal-linked sugar residue (GalNAc) was identified in Nature (40, 41), present in the outer core regions of LPS from *P. mirabilis* O27, *P. vulgaris* OX2, O4, and O8, and *P. penneri* strains 2, 4, 11, 17, 19, and 107. Biosynthesis of such compounds requires a novel class of enzymes that have not been identified so far. Secondly, a β-linked Kdo-residue was identified in the outer core region of *P. vulgaris* O25. Despite the fact that this is another case in which Kdo was identified in the outer core (which, again, rises questions regarding the biosynthetic pathways of such molecules), this Kdo residue is β-linked which was thought to occur only in capsular polysaccharides (50).

C-1-1-2-3. *Yersinia*

The core region of LPS from *Y. enterocolitica* O:8 was elucidated (51) (Table I) which is similar to the core structures of serotypes O:3 and O:9.

C-1-1-2-4. *Plesiomonas*

The core structure of LPS from *P. shigelloides*, a bacterium occurring in (fresh) water and wild and domestic animals, was investigated (52) (Table I). The core region is free of phosphate residues. Its novel feature is a Gal residue that is α-(1→4)-linked to Hep I. The linkage point of the O-specific polysaccharides was also determined.

の場合はそのO-4位にGlcNが置換する）で置換されている。しかし、serotype O3 LPSのコアではHep IIIのO-7位がβ-GalAで置換されている点が異なる。構造の最大の特徴は、GlcNのO-6位にL,D-Hep-(1→4)-Kdoが存在することである。この3糖はserotype O1、O2、O4、O5、O8、O12由来のS型LPSからも見つかっている(38)。serotype O1のR型LPSのコア構造（3糖L,D-Hep-(1→4)-Kdo-(2→6)-GlcNの存在についての研究はまだ行われていない）との違いは、serotype O1のS型LPSのコアにはD,D-ヘプトグリカンが見つかっていない点にある。R型では、S型で2糖L,D-Hep-(1→4)-KdoのGlcNのO-6位にヘプトグリカンが結合している。したがって*K. pneumoniae*のO1型では、S型、R型共にこの位置の置換が重要とみられる。

C-1-1-2-2. ***Proteus mirabilis***

プロテウス属の各種serotype由来LPSのコア領域については、非常に重要な研究が行われた(41-49)。構造はTable Iに示した。すべてのコアは、L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-[Kdo-(2→4)]-Kdoを共通な糖骨格として持っている。また、Hep IはO-4位にβ-Glc、Hep IIはO-3位にGalAとO-6位にPEをそれぞれ置換している。そして、この骨格にコア領域の特異性を決める様々な糖が結合している。

プロテウスのLPSのコアには2つの著しい特徴がある。一つは、*P. mirabilis* O27、*P. vulgaris* OX2、O4、O8、*P. penneri* の strain 2、4、11、17、19、107のLPSのコア領域に存在する、開環型アセタール結合糖残基(GalNAc)が天然に見つかったことである(40、41)。それぞれの成分の生合成は、これまでに見つかっていない新規の酵素を必要としている。二つめは、*P. vulgaris* O25の外部コアにβ結合のKdo残基が見つかったことである。外部コアにKdoが見つかったのは二例目であり、またこのような分子の生合成に問題があるにもかかわらず、このKdoは莢膜にしか存在しないと考えられていた(50) β結合である。

C-1-1-2-3. ***Yersinia***（エルシニア属）

Y. enterocolitica O:8のコア領域が解析され(51) (Table I)、serotype O:3およびO:9のコア領域と同じ構造であることがわかった。

C-1-1-2-4. ***Plesiomonas***（プレジオモナス属）

新鮮な水や野生動物、家畜に存在する細菌である *P. shigelloides* 由来LPSのコア構造が研究された(52) (Table I)。コア領域にはリン酸が存在しなかった。新規な特徴として、Hep Iにα-(1→4)結合したGalが見つかった。また、O抗原多糖の結合位置も決定された。

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C-1-2. *Neisseria* (Table II)

A few additional core structures from LPS of *N. gonorrhoeae* and *N. meningitidis* have been published in the past two years (53–56) which are summarized in Table II. A substitution by two PE groups that had earlier been found but not unequivocally attributed in *N. meningitidis* strain 6275 could be assigned to be linked to *O*-3 and *O*-6 of Hep II in the LPS from mutant BZ157 *galE* B5$^+$. Mutant BZ157 *galE* B5$^-$ possessed a Glc residue at position *O*-3. Strain BZ 157 shows phase variation, expressing most of the PE containing structures (56).

C-1-3. *Pasteurellaceae* (Table III)

C-1-3-1. *Haemophilus*

Further structures of core regions from LPS of *H. influenzae* and *H. somnus* have been published (57–61). As other cores published earlier, these core regions possess as common partial structure the tetrasaccharide L,D-Hep-(1→2)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo, in which Hep I is substituted at O-4 by β-Glc which in turn may carry other substituents in different strains, and Hep II carries 2-aminoethanol phosphate at O-6. In *H. influenzae* strains, only one Kdo residue is present that links the core region to lipid A which is phosphorylated. However, the core region of *H. somnus* strain 738 possesses instead an Kdo-(2→4)-Kdo disaccharide. Phosphocholine is present in most of the structures, substituting a Glc residue. Some of the core regions are partially *O*-acetylated.

C-1-2. *Neisseria*（ナイセリア属）(Table II)

この２年間に、Table II にまとめたように *N. gonorrhoeae*(淋菌)、*N. meningitidis* (髄膜炎菌) 由来 LPS のコア構造がさらに報告されている (53-56)。以前に *N. meningitidis* strain 6275 で見つかっていた結合位置不明の２つの PE が、BZ157 *galE* B5$^+$ 変異株において HepII のO-3とO-6位にアサインできた。BZ157 *galE* B5$^-$ 変異株は、O-3にGlc残基を持っていた。strain BZ157 は、相変化を示すが、そのほとんどはPE 含有の構造をしめす(56)。

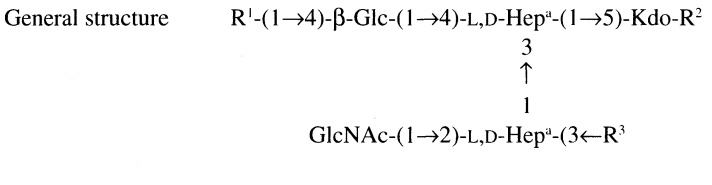
C-1-3. *Pasteurellaceae*（パスツレラ科） (Talbe III)

C-1-3-1. *Haemophilis*（ヘモフィルス属）

H. influenzae と *H. somnus* の LPS のコア領域の構造が発表された (57-61)。以前に発表された他のコアのように、このコア領域は L,D-Hep-(1→2)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo の共通三糖構造を持ち、さらに Hep I のO-4位に β-Glc またはその他の置換基が、Hep II のO-6位に PE が結合した構造をしていた。*H. influenzae* では、コア領域とリピドＡは１個のリン酸化された Kdo で結合されている。しかし、*H. somnus* strain 738 は、Kdo-(2→4)-Kdo 二糖をもっている。ホスホコリンは、ほとんどの構造に存在しており Glc に置換している。コア領域のあるものは、部分的に *O*－アセチル化されている。

Table II. The structures of core regions of LPS from *Neisseriaceae*.

General structure



Strain	R^1	R^2	R^3	*Ref.*
N. gonorrhoeae				
WG	β-GalNAc[b]-(1→3)-β-Gal[b]-(1→4)-β-GlcNAc-(1→3)-β-Gal-	n.d.	β-Gal-(1→4)-Glc-(1	(54)
N. meningitidis				
Serotype/Strain				
L1[c]	Neu5Ac-(2→6)-Gal-(1→4)-β-Gal	Kdo-(2→4)	PEA	(53, 6)

Unless stated otherwise, sugars are α-pyranosides (in case of *N. gonorrhoeae)* or α-D-pyranosides (*N. meningitidis*). [a] Absolute configuration not determined in the cores of LPS from *N. gonorrhoeae*. [b] Present in non-stoichiometric amounts. [c] *O*-Acetylated at unknown positions.

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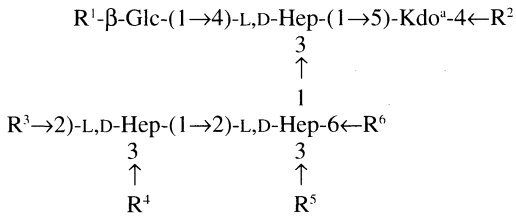
Table III. The structures of core regions of LPS from *Haemophilus*.

H. influenzae General structure



Strain	R^1	R^2	R^3	R^4	R^5	R^6	*Ref.*
Rd	PCho→6	PPE	β-GalNAc[b]-(1→3)-Gal[b]-(1→4)-β-Gal-(1→4)-β-Glc-(1	H	H	PE	(58)
RM.118-26	H	PPE	β-Gal-(1→4)-β-Glc[c]-(1	H	H	PE	(59)
RM7004[d]	β-Glc[b]-(1→4)-	P[e]	PCho[b]→6)-β-Gal-(1	H	H	PE	(60)
486	H	PPE	Ac	Neu5Ac-(2→3)-β-Gal-(1→4)-β-Glc-(1	PCho→6)-Glc-(1	PE	(61)
H. somnus 738	β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-Glc-(1→4)-L,D-Hep-(1→5)-Kdo 6 ↑ PCho; 3 ↑ 1 PE→6L,D-Hep; 4 ↑ 2 Kdo						(57)

Unless stated otherwise, sugars are α-D-pyranosides. PCho, phosphocholine. [a] Except for strain RM7004, this residue was identified to link the core region to GlcN II of lipid A. [b] Non-stoichiometric substitution. [c] Acetylated in non-stoichiometric amounts at *O*-6. [d] Possesses also core oligosaccharides that are *O*-acetylated at different but unknown positions. [e] Unspecified phosphate residue, probably at *O*-4.

C-1-4. *Pseudomonadaceae* (Table IV)

C-1-4-1. *Pseudomonas*

As found earlier in the core regions of from LPS of *P. aeruginosa*, two of the recently characterized structures (62–64) possess a GalN residue that is amidated by alanin and which substitutes (O-3 of) Hep II of the trisaccharide L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo. In the core region of LPS from mutant H4 the alanyl residue is replaced by an acetyl group. In all three characterized core regions, Hep II is further substituted at O-7 by a carbamoyl residue (65). Even more striking is, that the core regions are highly phosphorylated. In a cystic fibrosis isolate, the to date highest-phosphorylated core-lipid A backbone was identified (64). This core region possesses also *O*-acetyl groups.

C-1-4-2. *Burkholderia*

One core structure (of the LPS from *B. caryophylli*) was published recently (66) that possesses as unusual feature two L,D-Hep-(1→5)-Kdo moieties in the inner core region. This finding is of higher bearing in terms of biosynthesis, i. e. it is unclear whether or not the same heptosyltransferase is able to transfer both heptose residues.

C-1-4. *Pseudomonadaceae*（シュードモナス科） (Table IV)

C-1-4-1. *Pseudomonas*（シュードモナス属）

最近同定された *P. aeruginosa* (緑膿菌) 由来の LPS のコア領域は、以前に見つかっていたものと同様に、L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo の三糖を持ち、さらに Hep II のO-3位にアラニンアミド GlcN を結合した構造をしている (62-64)。H4 変異株由来 LPS のコア領域では、アラニンがアセチル基に置き換わっている。同定された３つのコア領域の Hep II のO-7位はカルバモイル化されている (65)。さらには、コア領域には多くのリン酸基が置換している。嚢胞性線維症からの臨床分離株が、コアーリピドA骨格のリン酸基置換が最も多い (64)。このコア領域は *O*–アセチル基を持っている。

C-1-4-2. *Burkholderia*（バークホルデリア属）

B. caryophylli 由来の LPS のコア構造が最近発表された (66)、内部コア領域に L,D-Hep-(1→5)-Kdo 部分を二つ持っていることがわかった。この発見は、同じヘプトシルトランスフェラーゼが両方の Hep を転移できるかどうか明かでないことなど、生合成の研究におおいに関連している。

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Table IV. The structures of core regions of LPS from *Pseudomonadaceae*.

Serotype/Strain	Structure	*Ref.*
Ps. aeruginosa		
PAO1	CONH$_2$ P P Ala | ↓ ↓ | 7 6 4 Glc-(1→6)-β-Glc-(1→3)-GalN-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[a] 4 2 4 ↑ ↑ ↑ 1 P 2 β-Glc-(1→2)-L-Rha-(1→6)-Glc Kdo	(62)
H4	CONH$_2$ P P | ↓ ↓ 7 4 2 GalNAc-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[a] 6 4 ↑ ↑ P 2 Kdo	(63)
2192[b]	CONH$_2$ P P Ala | ↓ ↓ | 7 4 2 Glc-(1→6)-β-Glc-(1→3)-GalN-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[a] 3 4 4 6 4 ↑ ↑ ↑ ↑ ↑ 1 1 P P 2 R[1] R[2] Kdo Either R[1] and R[2] are L-Rha and Glc or H and L-Rha-(1→6)-Glc, respectively.	(64)
Burkholderia caryophylli	Gal-(1→2)-Glc-(1→3)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo[a] 6 7 4 4 ↑ ↑ ↑ ↑ 1 1 1 2 Glc L,D-Hep β-Glc Kdo-(5←1)-L,D-Hep 6 ↑ 1 Glc	(66)

Unless stated otherwise, sugars are α-D-pyranosides. [a] This residue was identified to link the core region to GlcN II of lipid A. [b] The outer core region contains *O*-acetyl groups.

C-1-5. *Helicobacter* (Table V)

The core regions of LPS from various strains of *H. pylori* were published (67–70) that possess structures similar to the general structure of LPS core regions from this species, however, Hep I may carry at O-7 PE instead of phosphate. A novel core structure that lacks one of the D,D-Hep residues was found in strain J178.

C-1-6. *Bordetella* (Table VI)

The structure of the core region of LPS from *B. pertussis* was completed (71), and those from different strains of *B. hinzii* (72, 73) and *B. bronchiseptica* (72) were elucidated (Table VI).

C-1-5. *Helicobactor*（ヘリコバクター属） (Table V)

種々の *H. pylori*（ピロリ菌）由来 LPS のコア領域が、この種のLPS コア領域の一般構造と類似していることが発表された(67-70)。しかし Hep I のO-7位は PE のかわりにリン酸基を持っているようである。strain J178 は D,D-Hep の一つを持たない新規なコア構造であった。

C-1-6. *Bordetella*（ボルデテラ属） (Table VI)

B. pertussis（百日咳菌）(71) および、*B. hinzii* (72、73) と *B. bronchiseptica* (72) の異なる株由来 LPS のコア領域の構造が明ら

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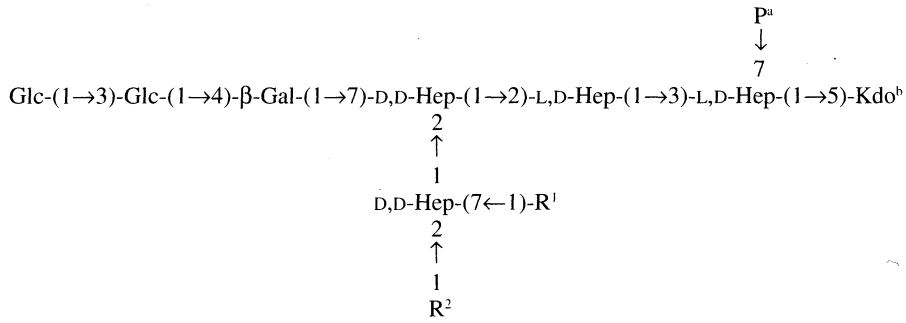
Table V. The structures of core regions of LPS from *Helicobacter*.

H. pylori General structure



Serotype/strain	R^1	R^2	*Ref.*
442, 471, F-58C, F-58A, J99, Sydney	*O*-chain	H	(67–69)
26695	*O*-chain	Glc-(1[→6)-Glc-(1]$_{1\text{-}2}$→6)-Glc-	(69)
J178		PE ↓ 7 Glc-(1→4)-β-Gal-(1→7)-D,D-Hep-(1→2)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo; 2 ↑ 1 β-GlcNAc	(70)

Unless not stated otherwise, sugars are α-D-pyranosides. [a] PE in strain Sydney. [b] This residue was identified to link the core region to GlcN II of lipid A.

All core regions share a similar carbohydrate backbone which is differently substituted in the side chains or with regard to phosphate residues.

C-2. Heptose-Deficient Core Regions

C-2-1. *Rhizobiaceae* (Table VII)

The structure of the core region of LPS from *R. etli* CE3 was completed (74).

C-2-2. *Chlamydia* (Table VII)

The structures of the core regions of native LPS from *Chlamydia trachomatis* serotype L2 and *Chlamydophila psittaci* strain 6BC were published (75, 76).

D. Concluding Remarks

In the past two years, there has been further progress in the structural elucidation of core regions of LPS obtained from various bacterial species, confirming that there is considerable structural variability in the core region. Still, most core regions

かになった (Table VI)。すべてのコア領域は同様な糖骨格を持っており、側鎖またはリン酸基の置換が異なっているだけであった。

C-2. ヘプトース欠損コア領域

C-2-1. *Rhizobiaceae* (Table VII)

R. etli strain CE3 由来 LPS のコア領域の構造が明かとなった。

C-2-2. *Chlamidia*（クラミジア） (Table VII)

C. trachomatis serotype L2 と *C. psittaci* strain 6BC 由来 LPS のコア領域の構造が発表された (75、76)。

D. 結　語

この 2 年間で種々の細菌由来 LPS のコア領域の構造解析がさらに進み、コア領域の構造多様性が明らかになってきている。ほとんどのコア領域は L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo の

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Table VI. The structures of core regions of LPS from *Bordetella*.

B. pertussis

```
                                                                                GalNA
                                                                                  1                    PPE[e]
                                                                                  ↓                      ↓
                                                                                  6                      4
GlcNAc-(1→4)-β-2,3-d-Man-2,3-NAcA[d]-(1→3)-FucNAcMe[c]-(1→6)-GlcN-(1→4)-β-Glc-(1→4)-L,D-Hep-(1→5)-Kdo[b]   (71)
                                                                  4                      3
                                                                  ↑                      ↑
                                                                  1                      1
                                                              L,D-Hep   GlcA-(1→2)-L,D-Hep-(7←1)-GlcN
```

Bordetella

```
                    GalNA
                      1                P[e]
                      ↓                  ↓
                      6                  4
R[1]-(1→6)-GlcN-(1→4)-β-Glc-(1→4)-L,D-Hep-(1→5)-Kdo[b]
          4                        3
          ↑                        ↑
          1                        1
          R[2]   GlcA-(1→2)-L,D-Hep-(7←1)-GlcN
                                   4
                                   ↑
                                  P[e]
```

hinzii ATCC 51730	R^1 = H and R^2 = H or β-FucN4N or partially *N*-acetylated Sug[f]-(1→3)-β-FucN4N	(72,73)
bronchiseptica 110H, Bp512	R^1 = L,D-Hep and R^2 = H or β-FucN4N or β-FucN4NMe or partially *N*-acetylated Sug[f]-(1→3)-β-FucN4N or Sug[f]-(1→3)-β-FucN4NMe	(72)

Unless stated otherwise, sugars are α-D-pyranosides. [a] Non-stoichiometric substitution. [b] This residue was identified to link the core region to GlcN II of lipid A. [c] FucNAcMe, methylacetamidofucosamin. [d] 2,3-d-Man-2,3-NAcA, 2,3-diacetamido-2,3-dideoxy-mannosaminuronic acid. [e] Product after deacylation. [f] Sug, 2,3-diamino-2,3,4-trideoxy-hex-4-enuronopyranosyl.

Table VII. The structures of core regions of LPS from *Rhizobium* and *Chlamydia*.

Rhizobium etli CE3

```
O-chain-(1→4)-Kdo-(1→6)-Gal-(1→6)-Man-(1→5)-Kdo[a]   (74)
                                 4          4
                                 ↑          ↑
                                 1          2
                               GalA   Kdo-(4←1)-GalA
                                            5
                                            ↑
                                            1
                                          GalA
```

C. trachomatis

```
Kdo-(2→8)-Kdo(2→4)-Kdo[a]   (75)
```

Chl. psittaci

```
Kdo-(2→8)-Kdo-(2→4)-Kdo[a]   (76)
            4
            ↑
            2
          Kdo[b]
```

Unless stated otherwise, sugars are α-D-pyranosides. [a] This residue was identified to link the core region to lipid A. [b] Non-stoichiometric substitution.

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comprise as partial structure the trisaccharide L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo, and if core structures from one bacterial family are compared, a common structural theme is often identified which may be varied in different species by different substituents. The common principle of LPS is represented by the core oligosaccharide and the lipid A, as proven by the findings that a) mutants which cannot synthesize a core are not viable and b) wild-type LPS may be S-form or R-form, thus, the expression of an O-specific polysaccharide is not a prerequisite for bacterial survival. Also, the binding of the core region to lipid A occurs always via a Kdo residue [except in two strains of *Acinetobacter* (6), where this Kdo is replaced in non-stoichiometric amounts by Ko]. A further general feature of LPS is that the core region is always negatively charged (provided by phosphoryl substituents and/or sugar acids like Kdo and uronic acids) which is believed to contribute to the rigidity of the Gram-negative cell wall through intermolecular cationic cross-links.

There are two types of core structures: those containing and those without heptoses. In the first type, L,D-Hep or D,D-Hep alone, or both may be present in a particular core structure. If present, D,D-Hep either decorates the inner core region (e.g. in *Y. enterocolitica*) or is attached to more remote parts of the carbohydrate chain. Still, the regulation of the distribution of L,D-Hep and D,D-Hep in the core region is not understood, and it is not known whether L,D-Hep and D,D-Hep are transferred by different transferases.

Finally, it should again be noted that, for the first time in Nature, an open-chain acetal-linked sugar residue (GalNAc) was identified (in the core region of *Proteus* LPS). The bearing of this novel structure for the biological functions of the core regions (antigenicity, stability of the cell wall, cell-cell interactions, virulence factors) as well as its biosynthesis remain to be elucidated.

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三糖部分構造を持っており、同一の細菌属間で比較すると、共通構造に種によって異なる置換基が付いていることが明らかになった。また、a) コアを合成できない変異株が生存できないこと、b) 野生型の LPS にはＳ型とＲ型が存在し、O 抗原多糖の発現は細菌の生存に必要ではないこと、から考えて LPS の重要な共通部分はコア多糖とリピドＡであるといえる。また、コア領域とリピドＡは常に Ｋｄｏ で結合している。例外は、*Acinetobactor* の２株 (6) で、Kdo が非量論量の Ko に置き換わっている。LPS のもっと一般的な特徴として、リン酸基および Kdo やウロン酸などの酸性糖によってコア領域に負電荷がかかっていることがある。このことが、正電荷との分子間相互作用を通してグラム陰性細菌の細胞壁の強固さに寄与していると考えられている。

コア構造には、ヘプトースを含むもの含まないものの２つの型がある。前者には、コアに L,D-Hep か D,D-Hep の一方を含むもの、または両方とも含むもののがある。D,D-Hep がある場合には、*Y. enterocolitica* の様に内部コアにあるか、または糖鎖のもっと離れたところについているかである。現在でも、コア領域における L,D-Hep と D,D-Hep の分布の制御については理解されておらず、 L,D-Hep と D,D-Hep が異なる酵素で転移しているかどうかについても分かっていない。

最後に、*Proteus* 由来 LPS のコア領域に開環型アセタール結合 GlcN 残基が自然界で初めて見つかったことをもう一度示しておく。新規の構造に関連して、コア領域の抗原性、細胞壁の安定性、細胞間相互作用、毒性因子といった生物学的役割や、その生合成など解決すべき問題が残っている。

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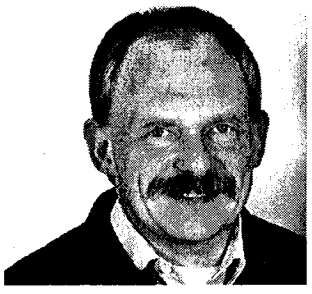
Profile of the Author



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