Chemical Structure and Biological Activities of Lipid A's from Various Bacterial Families

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Dedicated to Professor Dr. Otto Westphal on the occasion of his 65th birthday (February 1, 1978)

The endotoxic principle of lipopolysaccharides (LPS) is localized in their lipid A component. Biological effects of LPS on, for instance, body temperature, blood pressure, and blood picture, are also induced by free lipid A. In contrast to the great variability of the 0-specific chains, the chemical structure of lipid A is much more constant. It is common for *Salmonella* and similar for other genera of the Enterobacteriaceae. Recently, a number of lipid A's have been recognized that exhibited distinct structural features compared with Enterobacteriaceae. These lipid A's were found to be also distinct with regard to some of their biological properties.

It is now thirty years ago, shortly after the war, that Otto Westphal initiated a new approach to chemical and biological investigations of gram-negative bacterial endotoxins [1]. Three 'Westphal-specific' attributes determined this start: a tool, an idea, and a spirit.

The tool was the *phenol-water procedure,* which he had theoretically conceived and which had recently been practically elaborated [2, 3]. It provided a simple and efficient method for the extraction of biologically active bacterial polysaccharide constituents in a relatively pure form and high yield. The phenol-water procedure eventually proved to be not only generally applicable for the extraction of polysaccharides and lipopolysaccharides, but also for the isolation of glycopeptides and nucleic acids [4].

The idea and goal of the initial investigations was the isolation of the *bacterial product(s) responsible for the fever reaction* accompanying bacterial infections. Pyrogens were at that time believed to be contaminants of the bacterial endotoxins previously described. Thus, their purification was expected to lead to a pure fever-producing principle devoid of any toxic properties when introduced into higher animals and humans. Their eventual use in fever therapy was the aim of the investigations [5, 6].

The third factor, as important as the tool and the goal, was the spirit that guided the work, the *unique optimism, enthusiasm, and excitement,* which was continuously transferred to the co-workers. An expression of this spirit was, and remained, what was called the 'Westphal factor,' by which each step forward, however small, was multiplied, thus increasing the feeling of success and satisfaction – important factors in science.

This cluster of properties has continuously stimulated the work. The spirit was the root of the unique atmosphere in his growing Institute and has led to collaboration with numerous scientists throughout the world. This spirit projected by Otto Westphal has not changed over the years. Similarly, the major directions and goals remained the same with necessary modifications according to progress [7].

It was soon established that the product, extracted from gram-negative bacteria by phenol-water, contained a lipid component [5] and represented a *lipopolysaccharide* (LPS, Fig. 1) rather than a pure polysaccharide. It was then found that these LPS simultaneously exhibited *0 antigenicity* and *pyrogenicity,* and that they were endowed with all the known endotoxic properties. It was realized that a 'pure pyrogen' did not occur naturally in bacteria.

Chemical and structural investigations illustrated that the two biological characteristics of LPS, serological 0 specificity and endotoxicity, were expressed by separate regions of the molecule (Fig. 1). The 0-antigen factors (for instance, those of the Kauffmann-White

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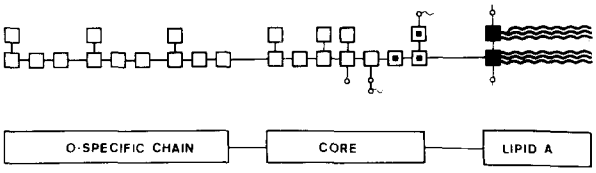

Fig. 1. Schematic representation of the structure of a *Salmonella* lipopolysaccharide. □ monosaccharide residue; ⊡ 2-keto-3-deoxyoctonate residue; ■ glucosamine residue; ↑ phosphate; ~ ethanolamine, ~~~ long-chain fatty acid residues

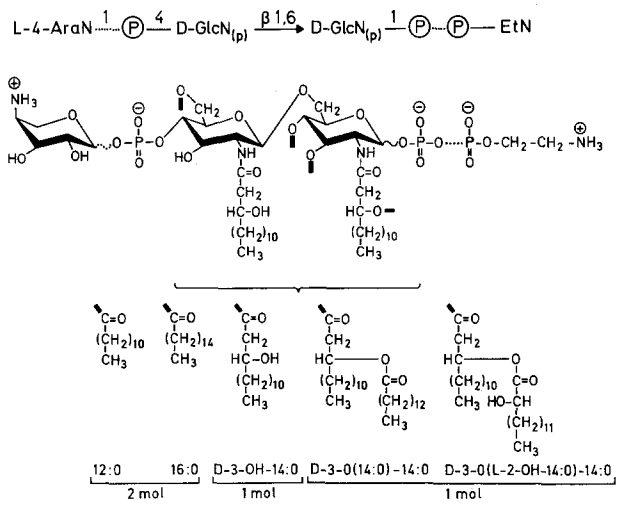

Fig. 2. Chemical structure of the lipid A of *Salmonella*. Dotted linkages indicate incomplete substitutions. The presence of a substituent linked to the hydroxyl group of amide-linked 3-hydroxymyristic acid is suggested, but the nature of the substituent is unknown. The pyranosidic (p) linkage of arabinosamine has not been established [13, 15–19]

scheme of *Salmonella* classification) are determined by oligosaccharide structures of the 0-specific chains [8, 9]. The endotoxic principle, on the other hand, was shown to be located in the lipid A region of LPS [10, 11]. Thus isolated polysaccharide or oligosaccharide fragments, devoid of lipid A, exhibit serological 0 specificity [9]; conversely, free lipid A, devoid of poly- or monosaccharide, exhibits endotoxic activities [11–14]. In the LPS molecule, the two regions, 0-specific chain and lipid A, are interlinked by the core oligosaccharide [11].

The great diversity of serologically distinct bacterial species corresponds to an equivalent diversity of structurally distinct 0 chains. Core structures are less variable when different genera are compared. The least variability, however, is shown by the lipid A structure. The expression of the known spectrum of biological activities associated with LPS appears to be consistent with chemical and structural prerequisites present in the structure of lipid A as identified for Enterobacteriaceae [11].

Lipopolysaccharides with atypical lipid A structures do exist, however, and, as was recently reported, bacterial groups remote from Enterobacteriaceae may contain a structurally quite distinct lipid A. As might be expected, they are devoid of a number of the characteristic endotoxic activities.

The following sections will describe the structure of *Salmonella* lipid A and its biosynthetic pathway, the structures of lipid A's distinct from that of *Salmonella,* and some biological activities exhibited by different lipid A's.

Lipid A Structures

Salmonella Lipid A

The structure of *Salmonella* lipid A (Fig. 2) contains a central backbone of a β1,6-linked D-glucosamine disaccharide [15] substituted at positions 4′ and 1 by phosphomonoester residues [16]. The hydroxyl group at C3′ functions as the attachment site of the core. Long-chain fatty acids linked to the amino and hydroxyl groups of the disaccharide confer the lipophilic properties to the lipid A molecule. The amino groups are substituted by D-3-hydroxymyristic acid, and the available hydroxyl groups by lauric, palmitic, and two molecules of D-3-hydroxymyristic acid. Part of the latter (1 molecule) is substituted by myristic acid [17] and to a smaller extent by L-2-hydroxymyristic acid [18]. Recent results indicate the possible involvement of 3-hydroxymyristic acid in a substitution of amide-linked 3-hydroxymyristic residues [13].

Another form of intrinsic microheterogeneity in lipid A is caused by substituents linked to the phosphate groups [13, 19]. The phosphate groups of most lipid A molecules are unsubstituted. In a variable number of molecules though, probably depending on culture conditions, the phosphate groups at positions 4′ and 1 are substituted by 4-amino-L-arabinose and phosphorylethanolamine residues, respectively [19]. Neither their amino nor their hydroxyl groups are acylated.

In the past, structural studies on lipid A were performed either on free lipid A, obtained by mild acid hydrolysis, or on bound lipid A, that is, on the complete LPS. The failure to isolate mutants by using the usual phage-selection methods, defective in the KDO*-lipid A region, indicated that the KDO-lipid A region is indispensible for the viability of the bacteria.

* KDO, 2-keto-3-deoxyoctonate

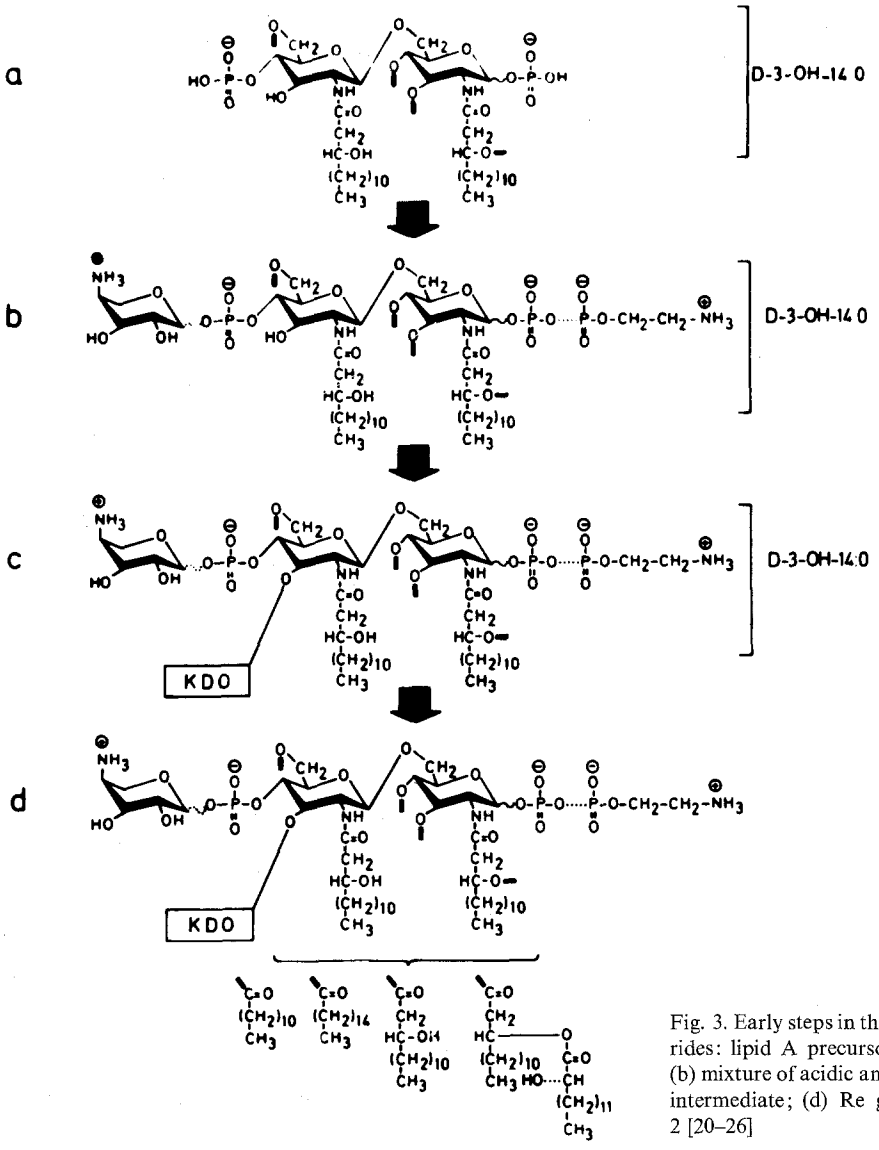

Fig. 3. Early steps in the biosynthesis of *Salmonella* lipopolysaccharides: lipid A precursor molecules. (a) Acidic lipid A precursor; (b) mixture of acidic and neutral lipid A precursor; (c) hypothetical intermediate; (d) Re glycolipid. Compare also legend to Figure 2 [20–26]

Recently, however, a new class of LPS-defective mutants was recognized, carrying a temperature-sensitive defect in the synthesis of KDO [20–24]. At low temperature (28 °C), KDO and, hence, normal LPS are synthesized. At the nonpermissive temperature (42 °C), no KDO is made and the mutants cease to synthesize LPS. Growth continues, but only for one or two generations, during which time the mutant accumulates an incomplete lipid A. This lipid A precursor molecule has been designated as 'acidic' due to its behavior on DEAE cellulose. It contains the diphosphorylated β1,6-linked diglucosamine backbone, which is substituted by ester- and amide-linked 3-hydroxymyristic acid [22, 23] (Fig. 3a). As expected, it does not contain KDO. Surprisingly, it also lacks the nonhydroxylated fatty acids, lauric, myristic, and palmitic acid.

In further studies a second, neutral lipid A precursor [25] was identified which is formed by the mutants at semipermissive temperature (34 °C) in addition to the acidic precursor. Like complete lipid A, the neutral precursor carries the polar head groups, 4-aminoarabinose and phosphorylethanolamine [19, 25] (Fig. 3b). Both precursor molecules are found in the inner membrane and are transported only to a small extent to the outside. Pulse-chase experiments have shown that the pulse labels acidic precursor synthesized at the high temperature and that the label, following a shift to the low temperature, is chased (partly) via neutral precursor into LPS. From these

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and other data, the early steps of LPS biosynthesis may be visualized [25, 26] (Fig. 3). The acidic precursor is partly converted to neutral precursor, then KDO (two or three molecules) is transferred, and finally the Re LPS is completed by the addition of the nonhydroxylated fatty acids (and, possibly, more KDO).

Structurally, the acidic precursor (with some hydroxyl groups being esterified, some free) holds a position between free lipid A (all hydroxyl groups acylated), alkali-, methylate-, or hydroxylamine-treated lipid A (all hydroxyl groups free), and hydrazine treated lipid A (hydroxyl and amino groups free).

It is clear from the above results that Re LPS is the first structure in LPS biosynthesis containing complete lipid A.

Lipid A of Enterobacteriaceae

Although only *Salmonella* lipid A has as yet been studied in detail, studies on lipid A of other enterobacterial genera have revealed a great structural similarity in this family. They all contain the β1,6-linked D-glucosamine disaccharide substituted by phosphate groups at C1 and probably C4′, and carrying amide-bound D-3-hydroxymyristic acid [11, 13, 43]. Some differences compared with *Salmonella* have, however, been detected so far. As shown in Table 1, they concern the presence or the quantity of 4-aminoarabinose, phosphorylethanolamine, and L-2-hydroxymyristic acid.

Table 1. Lipid A of Enterobacteriaceae [43]. A common feature is the backbone of a diphosphorylated β1,6-linked D-glucosamine disaccharide with the amide-bound D-3-hydroxymyristic acid. The degree of substitution may be (p) partial or (c) complete (n.d., not determined). The backbone of *Klebsiella* has not been identified

Lipid A from	4-Amino-L-arabinose	Phosphoryl-ethanolamine	L-2-Hydroxy-myristic acid
Salmonella	+ (p)	+ (p)	+
E. coli	−	n.d.	−
Shigella	−	n.d.	−
Proteus	+ (c)	−	−
Yersinia	+ (p)	n.d.	−
Serratia	n.d.	n.d.	+
Klebsiella	n.d.	n.d.	+

Other Lipid A's

A number of randomly selected lipid A preparations of different origin when analyzed comparatively for their building blocks were shown to differ compositionally from *Salmonella* lipid A (Table 2). Additional D-glucosamine [27], D-arabinose [28], D-mannose [29], galactosamine [30], and in some cases only traces of phosphate were detected. The lipid A's of *Rhodopseudomonas viridis* (and *Rh. palustris*) contain 2,3-diamino-D-glucose instead of glucosamine [31, 32]. The structures of some of these lipid A's have been studied.

Chromobacterium violaceum lipid A [27] (Fig. 4) contains a central phosphorylated β1,6-linked diglucosamine backbone with 4-amino-L-arabinose as one substituent. To this extent it is similar to lipid A of *Salmonella*. In contrast to *Salmonella* lipid A, however, the phosphate bound to C1 of the reducing glucosamine carries a D-glucosamine residue instead of phosphorylethanolamine. Both head groups are present in molar ratios. The structure of this lipid A represents an unusual nonreducing tetrasaccharide of amino sugars with phosphodiester bridges. Only the central disaccharide is acylated, being substituted with D-3-hydroxylauric acid in amide linkage, and lauric, palmitic, L-2-hydroxylauric, and D-3-hydroxycapric acid in ester linkage. Mild acid hydrolysis of the LPS leads to the liberation of the polysaccharide component. Glucosamine-1-phosphate and 4-aminoarabinose are also liberated, and a *Salmonella*-like free lipid A is obtained (Fig. 5).

Table 2. Chemical composition of lipid A's derived from different bacterial groups

Lipid A	Composition[a]					
Salmonella [15, 17, 19]	GlcN-β1,6-GlcN	P	FA	AraN	P-EtN	
Chromobacterium violaceum [27]	GlcN-β1,6-GlcN	P	FA	AraN	GlcN	
Rhodospirillum tenue [28]	GlcN-β1,6-GlcN	P	FA	AraN	Ara	GlcN
Rhodopseudomonas viridis [31, 32]	2,3-diamino-D-glucose			FA		
Chromatium vinosum [29]		GlcN	Man	FA	(P?)	
Moraxella duplex [30]		GlcN	GalN	FA	(P?)	
Neisseria catarrhalis [30]		GlcN	GalN	FA	(P?)	

[a] GlcN, D-glucosamine; GalN, galactosamine; AraN, 4-amino-L-arabinose; Man, D-mannose; Ara, D-arabinose; FA, fatty acids; EtN, ethanolamine; P, phosphate

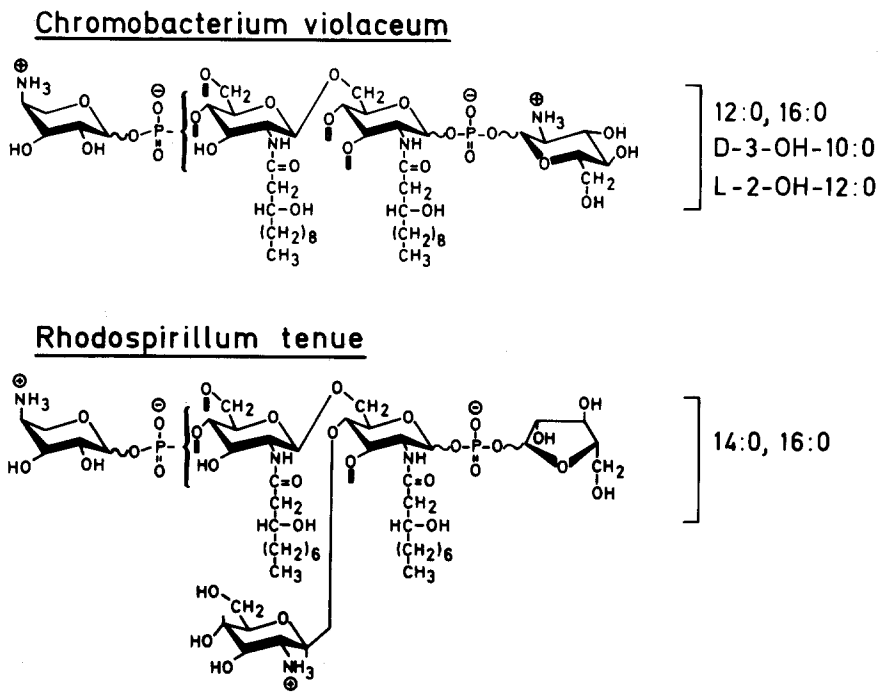

Fig. 4. Chemical structure of the lipid A's of *Chromobacterium violaceum, Rhodospirillum tenue,* and *Rhodopseudomonas viridis.* The attachment sites to the nonreducing glucosamine of KDO and phosphate are unknown for both, *Ch. violaceum* and *Rh. tenue* [27, 28, 31, 32]

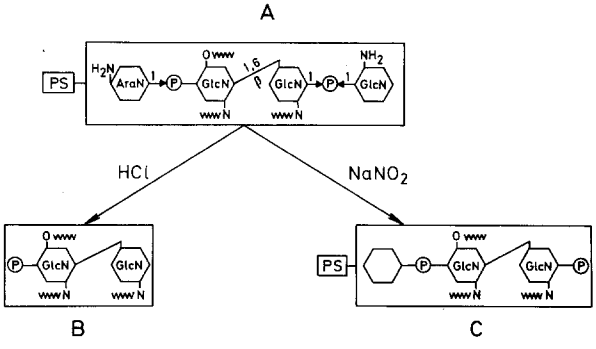

Fig. 5. Chemical degradation of the lipopolysaccharide of *Chromobacterium violaceum.* Mild acid treatment degrades the lipopolysaccharide (A) and the following products are formed: aminoarabinose, glucosamine phosphate, free polysaccharide, and free *Salmonella*-like lipid A (B). Treatment with nitrite removes the glucosamine head group as anhydromannose, and produces an altered LPS (C) whose lipid A component now resembles that of *Salmonella* [27]. The simplified scheme shows only one ester-bound fatty acid

As shown in Figure 5, deamination of the LPS of *Chromobacterium violaceum* removes the glucosamine head group (and modifies 4-aminoarabinose in an unknown way), yielding a LPS whose lipid A component is altered and again resembles the lipid A of *Salmonella* [27].

Rhodospirillum tenue lipid A (Fig. 4) represents a pentasaccharide structure of D-glucosamine, 4-amino-L-arabinose, and D-arabinose with phosphodiester bridges [28]. Its central moiety forms a branched glucosamine trisaccharide. The phosphorylated β1,6-linked glucosamine disaccharide, being identical to the *Salmonella* lipid A backbone, carries D-3-hydroxycapric acid in amide linkage and myristic and palmitic acid in ester linkage. D-Arabinose is present in the furanosidic form. Treatment with mild acid liberates 4-aminoarabinose, arabinose-1-phosphate, arabinose, and phosphate, leaving *Salmonella*-like lipid A [28].

Rhodopseudomonas viridis lipid A (Fig. 4) contains 2,3-diamino-D-glucose with the amino groups partly substituted by D-3-hydroxymyristic acid and the hydroxyl groups free [31, 33]. This structure is different from all other lipid A's so far analyzed.

Biological Activities Exhibited by Different Lipid A's

Immunological Cross Reactions

Two different test systems have been applied in order to demonstrate serological cross reactivity of the different lipid A's with *Salmonella* lipid A. In the passive hemolysis test (in vitro), anti-*Salmonella* lipid A antiserum is reacted with sheep red cells coated with homologous or heterologous free lipid A in the presence of complement [34]. In a variation of this test, a homologous *Salmonella* lipid A hemolytic system can be inhibited by preincubation of the antiserum with heterologous lipid A's. Free lipid A, but not LPS-bound lipid A, can be detected in these test systems.
In a second (in vivo) test [35], free lipid A and bound lipid A as present in LPS can be tested for cross reactivity. Rabbits receive a pyrogenic dose of lipid A or LPS on day 0, anti-*Salmonella* lipid A antiserum on day 1, and another pyrogenic dose of free lipid A or LPS on day 2. In homologous or heterologous systems, provided a serological cross reaction exists between the pyrogen and the lipid A antiserum, the fever reaction on day 2 is suppressed. This suppression has been shown to be lipid A-specific [11, 35].
Table 3 shows that of the LPS preparations listed only those from *Salmonella* show cross reactivity. This is due to the structural identity of their lipid A component. Other enterobacterial LPS (e.g., *E. coli, Sh. flexneri*) also cross react with the anti-*Salmonella* lipid A serum [14, 35]. The LPS of *Ch. violaceum* and *Rh. tenue* exhibit no cross reaction with anti-*Salmonella* lipid A serum [35, 36]. Cross reactions are, however, seen with the free lipid A's from both; this is probably due to the removal of glucosamine-1-phosphate and arabinose, respectively, which leads to a *Salmonella*-like lipid A structure [27, 28, 36]. Similarly, *Ch. violaceum* LPS after degradation with $NaNO_2$ [27] (Fig. 5) exhibits serological cross reactivity with *Salmonella* lipid A antiserum (in the in vivo test [35]). No lipid A-dependent serological cross reaction is seen between the LPS and free lipid A of *Salmonella* and *Rhodopseudomonas viridis* [36]. Similarly, it has been shown that antiserum raised against *Rh. viridis* lipid A reacts with free lipid A of *Rh. viridis* and *Rh. palustris,* but does not cross react with *Salmonella* free lipid A [36].

Complement Activation

The capacity to activate complement is a well-known property of most LPS. In this test, LPS is incubated in the absence of added antibody with complement (in general, guinea pig serum) and the reduction in hemolytic units is determined [37]. Recent studies with enterobacterial LPS have led to the following conclusions [11, 37, 38]:
(1) Most LPS, S and R forms, are highly active in reacting with complement provided they are present in a state of high aggregation, e.g., in the original form as obtained from the bacteria, or after their conversion into a uniform salt form (sodium or calcium).

Table 3. Biological activities of lipopolysaccharides and free lipid A's derived from different bacterial groups [36]

	Salmonella spp.		*Ch. violaceum*		*Rh. tenue*		*Rh. viridis*	
	LPS	Lipid A	LPS	Lipid A	LPS	Lipid A	LPS	Lipid A
Immunological cross reactivity with anti-*Salmonella* lipid A antiserum[a]	+[e]	+	−	+	−	+	−	−
Reactivity with complement[b]	+(−)	+	−	−	−	−	+	+
Pyrogenicity[c]	+	+	+	+	±	±	−	−
Lethal toxicity[d]	+	+	+	+	−	+	−	−

[a] Test systems for free lipid A: inhibition of passive hemolysis [34], test system for LPS and free lipid A: suppression of pyrogenicity [35] (see text)
[b] The test system has been described in [37]. Maximum reduction in complement hemolytic activity, +: ≧80%; −: ≦30%
[c] Test system: fever reaction in rabbits, determination of the MPD-3. +: ~0.01 µg; +−: ~0.1 µg; −: ≧1 µg per rabbit [35, 36]
[d] Test system: determination of the LD_{50} in adrenalectomized mice. +: ~0.01 µg; −: ≧10 µg per mouse [14]
[e] LPS of *E. coli* and *Sh. flexneri* also cross react.
(MPD-3: minimum pyrogenic dose raising the body temperature of rabbits by 0.6 °C 3 h after injection. LD_{50}: dose that kills 50% of adrenalectomized mice)

(2) Lipopolysaccharides present in a disaggregated state, e.g., in the form of their triethylamine salt, do not react with complement.
(3) A number of LPS have been encountered that do not react with complement (in the absence of antiserum) no matter in which salt form or state of aggregation they are present. *S. minnesota* Ra and Rb LPS are examples.
(4) Free enterobacterial lipid A is highly active in complement activation no matter whether the parent LPS belongs to the active or inactive class of LPS and irrespective of the salt form (due to the fact that free lipid A, in contrast to LPS, is rapidly converted in serum into a salt form of high aggregation).
Table 3 shows the complement-activating capacity of the investigated LPS and free lipid A's. No complement activity is seen with the LPS of *Ch. violaceum* and *Rh. tenue*. As outlined above, inactive LPS also occur in *Salmonella*. It was surprising, however, to find that in the case of *Ch. violaceum* and *Rh. tenue* also the corresponding free lipid A's did not react with complement, although being potent pyrogens and lethal toxins (see below). In contrast, it was found that although inactive as endotoxins (see below), both LPS and free lipid A of *Rh. viridis* exhibit high reactivity toward complement [36].

Pyrogenicity and Lethal Toxicity

Pyrogenicity is measured in rabbits where the MPD-3 (minimal pyrogenic dose raising the core temperature by 0.6 °C, 3 h after injection) is in the range of 10 ng/rabbit for LPS or free lipid A [35]. Lethal toxicity is determined in mice, made susceptible to the action of endotoxin by adrenalectomy. The LD_{50} of *Salmonella* LPS and free lipid A is about 10 ng/mouse [14].
Table 3 shows [36] that LPS and free lipid A from *Ch. violaceum* behave as typical endotoxins, i.e., they are pyrogenic and express lethal toxicity. Both LPS and free lipid A from *Rh. tenue* are weak pyrogens; although the LPS is nontoxic, the isolated free lipid A exhibits lethal toxicity. On the other hand, with *Rh. viridis*, neither pyrogenicity nor lethal toxicity is seen with either LPS or free lipid A.

Biological Activities of Lipid A Precursor and Chemically Degraded Lipid A

The results of Table 4 show that the precursor exhibits serological lipid A specificity, mitogenicity, and lethal toxicity. It is a weak pyrogen and shows questionable reactivity toward complement. De-O-acylated lipid A is serologically active and a potent mitogen devoid of pyrogenicity and lethal toxicity. De-O,N-acylated lipid A is inactive in all tests.

Table 4. Biological activities of complete *Salmonella* lipid A, lipid A precursor and chemical degradation products. For test systems see Table 3. Mitogenicity was determined according to [42]. Alkali- and hydrazine-treated *Salmonella* lipid A have been described previously [15, 17]

	Complete lipid A	'Acidic' precursor	De-O-acylated lipid A	De-O,N-acylated lipid A
Cross reaction with anti-*Salmonella* lipid A antiserum in vitro	+	+	+	−
Complement reactivity	+	±	−	−
Mitogenicity	+	+	+	−
Pyrogenicity	+	±	−	−
Lethal toxicity	+	+	−	−

Concluding Remarks

The structures of the lipid A's from *Ch. violaceum* and *Rh. tenue* (Fig. 4) resemble each other and that of lipid A from *Salmonella* (Fig. 2) in that they contain a basal backbone of a diphosphorylated diglucosamine that in *Salmonella* is partially, and in the other two species completely, substituted on the phosphate group linked to the nonreducing glucosamine by 4-aminoarabinose (no arabinosamine is found in *E. coli* lipid A). These central structures are substituted on the phosphate group at C1 by different substituents: a partial substitution by phosphorylethanolamine is characteristic for *Salmonella* [19], a complete substitution by glucosamine for *Ch. violaceum* [27] and by arabinose for *Rh. tenue* [28]. The linkages of these substituents are acid-labile and are at least partly cleaved when lipid A is liberated from the respective LPS. The structures of free lipid A from *Salmonella* and *Ch. violaceum*, therefore, resemble each other, and that of *Rh. tenue* should be distinct only by the additional glucosamine residue, glycosidically linked to the glucosamine backbone.
With these structural relationships in mind, one is tempted to explain differences in biological activities as exhibited by the various LPS and the corresponding lipid A's. The finding that *Ch. violaceum* and *Rh. tenue* LPS do not cross react with anti-*Salmonella* lipid A antiserum, but that the respective free (and partially degraded) lipid A's do, could indicate that the substituent carried by the phosphate group at C1 prevents lipid A antibodies from interaction with the serological determinant structure of lipid A. It would follow that the immunodominant area in lipid A is near to the inhibiting head groups. Recent findings seem to agree with this hypothesis. It has been shown that the hydroxamate of 3-hydroxymyristic acid is a good inhibitor of the *Shigella sonnei* lipid A/anti-lipid A system [40]. Furthermore, degradation prod-

ucts of *Salmonella* lipid A are effective inhibitors only if they contain 3-hydroxymyristic acid in amide linkage to at least one of the glucosamine residues [41].

At present, structural requirements for the interaction of an LPS with complement are not known. It is therefore not possible to indicate why both LPS and free lipid A of *Ch. violaceum* and *Rh. tenue* are inactive in this test, while both preparations from *Rh. viridis* are highly active. In previous studies, evidence for the existence of a correlation between reactivity toward complement and the phosphate content of the LPS had been found. Phosphate-rich LPS such as *S. minnesota* Ra and Rb (whose free lipid A, however, was active) were complement-inactive [11], whereas phosphate-poor LPS such as *Anabaena variabilis* and *Chromatium vinosum* were active (Mayer and Galanos, unpubl.).

It is of interest to note that LPS-induced complement activation is also seen in vivo [14, 39]. In rats, the application of LPS in uniform salt forms led to the recognition of two independent depressions in complement activity, an early depression appearing almost immediately after injection of LPS, and a later one developing gradually during 6–9 h after injection. The early depression is caused by high molecular weight LPS preparations which also exhibit in vitro complement activity. This depression is not related to the endotoxic properties of the LPS. The late depression of complement is induced only by endotoxically active LPS, this being independent of their molecular weight or their in vitro complement activity. The complement-active but nontoxic LPS of *Rh. viridis* induces in vivo only the first depression [39].

Ch. violaceum LPS and free lipid A are potent pyrogens and lethal toxins. Most interesting was the finding that with the *Rh. tenue* preparations pyrogenicity and lethal toxicity are separable: the LPS is moderately pyrogenic and nontoxic; the free lipid A, although weakly pyrogenic, is highly toxic. An explanation can presently not be given, but weak pyrogenicity coupled with high toxicity has also been found with the acidic lipid A precursor [11]. The neutral precursor has not yet been investigated regarding its biological activity.

The lipid A from *Rh. viridis* is structurally so different from *Salmonella* lipid A that the absence of endotoxic activities is not surprising. Presently it is not known whether this situation is similar to that of *Brucella* LPS, which are of considerably low endotoxic activity in normal test animals, but become highly active in animals sensitized by pretreatment with *Brucella*.

It is hoped that further chemical and biological investigations of different types of lipid A's will reveal relationships existing between chemical structure and biological activity.

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