Three Dimensional Structure of the Carbohydrate Moiety of a Lipopolysaccharide. Computer Calculations.

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Lipopolysaccharide, Carbohydrate Moiety, Structure

The cell wall of the mutant R 595 of *Salmonella minnesota* contains a lipopolysaccharide in which only a trisaccharide consisting of three KDO* residues is linked to a lipid termed lipid A. Considering the sterical requirements of fourteen rotation angles, we have calculated allowed conformation of this trisyccharide unit and its linkage to lipid A. The calculations have been based on averaged crystallographic data of pyranose rings, ester-, N-acetyl-, phosphate- and carboxylgroups.

Because of considerable sterical hindrance, there are unique positions for the rotation around the axes O_3-C_3 of the glucosamine residue and O_1-C_1 of KDO residue 1 as well as for the rotation of the N-acetylgroup at the C_2-atom of glucosamine. Similarly, the rotation angle of the carboxylgroup on KDO residue 1 and the rotation angles of the phosphategroup linked to glucosamine are highly restricted, while a large range of angles is allowed for the bond of the ester group to glucosamine.

Chemical sequence analysis yields two possibilities for the linkage between the KDO residues 1 and 2. Linkage of O_4 of KDO 1 in equatorial position is restricted to a narrow range of angles, whereas the linkage to O_4 in axial – and to O_3 in axial and equatorial position is unfavourable. Furtheron chemical sequence analysis suggests two ways how to link KDO residue 1 and 3. The linkage to the oxygen atom on C_7 of KDO residue 1 can be described by four, the linkage to the oxygen on C_6 with three rotation angles. In either case two of the rotations are highly restricted. In the first case the two remaining angles have large rotational freedom, while the second case is sterically unfavorable.

The feasibility of the computer calculations has been demonstrated by the construction of a three-dimensional atomic model.

Introduction

The cell walls of Gram-positive bacteria consist mainly of murein combined with polysaccharides, teichoic- and teichuronic acids [1], whereas the cell walls of Gram-negative bacteria consists of a thin murein layer superposed by lipoproteins, proteins and lipopolysaccharides [2–4]. The lipopolysaccharides form the outer layer of the cell walls, without being covalently linked to the rest. Therefore they can readily be extracted with detergents and phenol [5]. In case of bacterial infections, lipopolysaccharides or endotoxins are secreted into the organism, where they are highly toxic, causing fever and septic shock in addition to immunological reactions.

To find a structure-function relationship for this medically important substance, the chemical structures of the lipopolysaccharides of several kinds of bacteria have been investigated [6, 7]. All of them are based on a similar structural principle.

The chemical composition of the lipopolysaccharide of *Salmonella minnesota S* [7] is shown in Fig. 1. The O-specific polysaccharide characteristic for *Salmonella minnesota S* and its serological reactions is linked to the core polysaccharide common to all species of Salmonella. The core itself is linked through its KDO trisaccharide to a lipid component termed lipid A, which is similar in all lipopolysaccharides. Lipid A of *Salmonella* consists of two β-1,6-linked glucosamine residues, each of which contains a phosphate residue to which a phosphorylethanolamine or a 4-amino-L-arabinose residue may be linked [8]. The hydroxylgroups of the glucosamine residues are esterified with lauryl-, palmityl-, and D-3 myristomyristic acid residues respectively, while the aminogroups are substituted by D-3-hydroxymyristic acid [9, 10].

* KDO = 2 Keto-3-deoxyoctonate. The carboxyl C-atom of KDO is denoted C_0. The following atoms have been numbered from 1 to 7. In this case numbers 1 to 6 are identical with the usual numbering in pyranose ring shugars.

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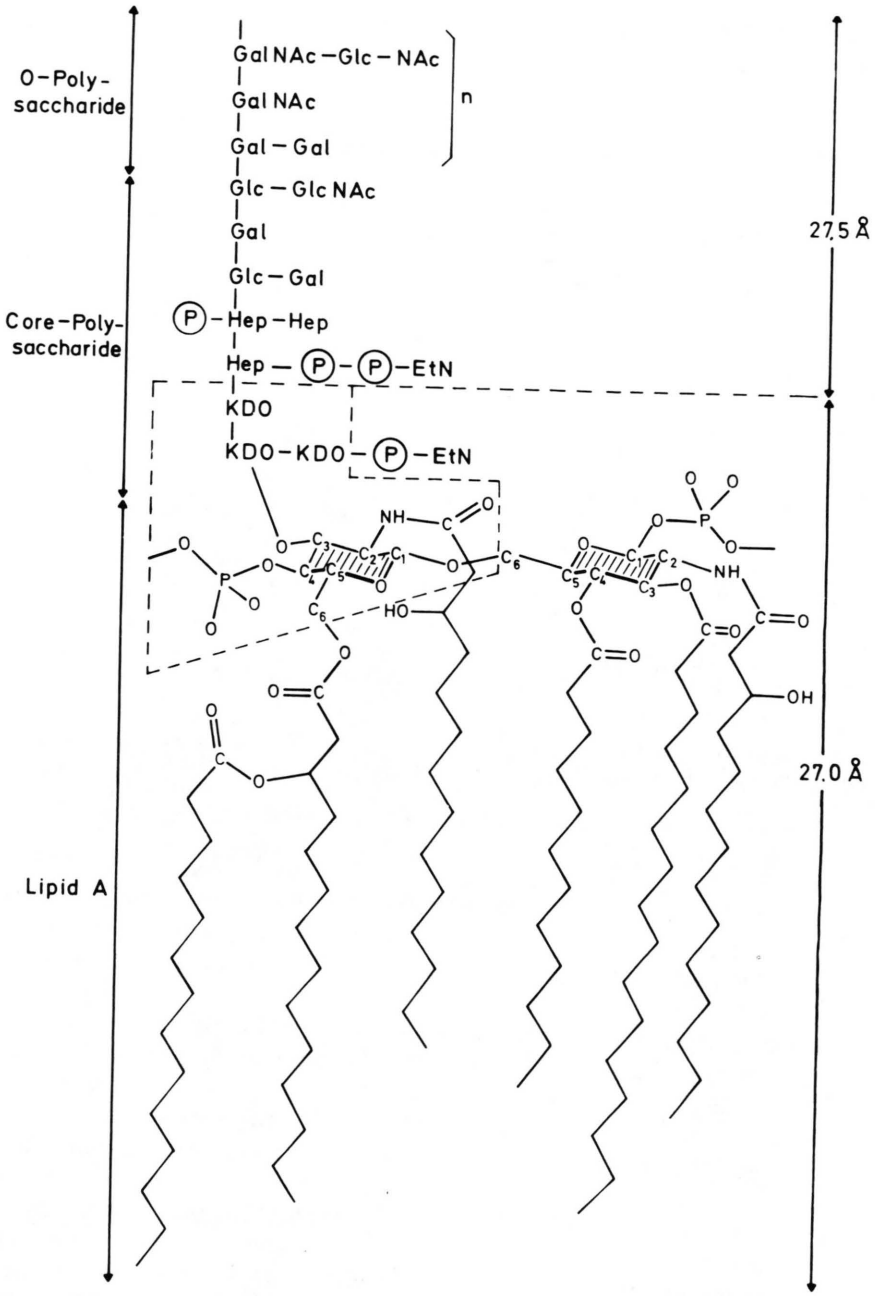


Fig. 1. Diagrammatic representation of the lipopolysaccharide of *Salmonella minnesota S* ([7] and E. Th. Rietschel personal communication). KDO = 2 keto-3-deoxyoctonate; Hep = L-glycero-D-mannoheptose; Glc = glucose; GlcNAc = N-acetylglucosamine; Gal = galactose; GalNAc = N-acetylgalactosamine; Ⓟ – EtN phosphoethanolamine; Ⓟ – Ⓟ – EtN = pyrophosphoethanolamine.

Since the lipopolysaccharides by themselves cause similar toxic reactions (pyrogenicity, septic shock), it seems important to resolve their tertiary structure in addition to their chemical composition in order to find an explanation for their structure-function relationship.

Results of electron microscopy and X-ray diffraction on lipopolysaccharides [11] can be interpreted by a bilayer structure in an aqueous medium, with the hydrophobic hydrocarbon layers attached to each other. The following results have been obtained by comparing X-ray diffraction data from dried specimens of both *Salmonella minnesota* R 595 and *Salmonella minesota S* [12]. The monolayer of *Salmonella minnesota* R 595 with three KDO residues linked to lipid A has a thickness of 27 Å, while the complete lipopolysaccharide of *Salmonella minnesota S* (Fig. 1) is 54.5 Å thick. In either case the fatty acid chains of the lipopolysaccharides are hexagonally packed with a periodicity of 4.1 Å.

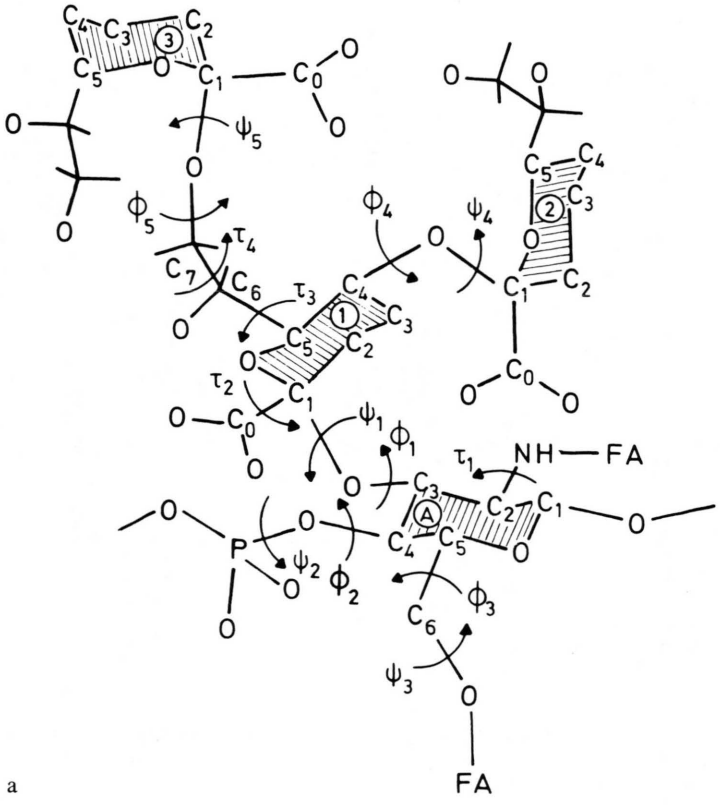
Since the membran-forming lipopolysaccharide does not crystallize, a better resolution of its structure cannot be obtained by X-ray methods. Based on the known chemical composition, however, allowed conformations for its carbohydrate moiety could be calculated, when fourteen rotation angles were considered for the carbohydrate moiety of the lipopolysaccharide in the cell wall of the mutant *Salmonella minnesota* R 595 (Figs. 1 and 2). The result is almost unique because of considerable sterical restrictions.

Methods

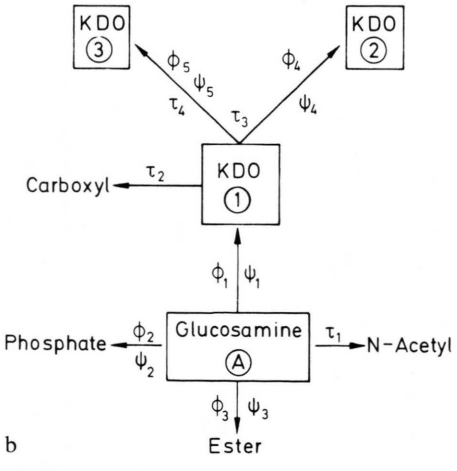
To yield highly reliable atomic coordinates, crystallographic data of hexoses (in glucopyranosidic conformation), ester-, N-acetyl-, phosphate-, and carboxylgroups have been transformed to common rectangular coordinate systems and averaged.

Atomic coordinates of the carbohydrates

Table I gives the averaged atomic coordinates of fourteen hexoses in the glucopyranosidic conformation [13–23]. The C_3-atoms are shifted into the



a



b

Fig. 2a. Structure formula and rotation angles of the carbohydrate moiety of a lipopolysaccharide containing three KDO residues linked to lipid A (see framed part of Fig. 1). Ⓐ = N-acetylglucosamine residue. ① ② ③ =KDO residues. FA = fatty acid residue. In our calculations, only the α-carbon atoms of the hydrocarbon chains of fatty acid residues are considered.

Fig. 2b. Simplified scheme of Fig. 2a.

Table I. Atomic coordinates of hexose in glucopyranosidic conformation.

C_1	1.35	O_1 axial	1.32	O_1 equatorial	1.46	H_1 axial	1.39	H_1 equatorial	1.38
	2.10		2.22		3.37		2.12		3.01
	0.00		1.39		− 0.51		0.98		− 0.37
C_2	0.05	O_2 axial		O_2 equatorial	− 1.04	H_2 axial	0.00	H_2 equatorial	− 0.78
	1.45				2.16		1.52		1.99
	− 0.46				0.16		− 1.44		0.01
C_3		O_3 axial	0.00	O_3 equatorial	− 1.16	H_3 axial	− 0.03	H_3 equatorial	− 0.82
			0.00		− 0.64		− 0.02		− 0.45
			1.41		− 0.51		0.98		− 0.36
C_4	1.24	O_4 axial	1.21	O_4 equatorial	1.28	H_4 axial	1.21	H_4 equatorial	1.27
	− 0.75		− 0.96		− 2.04		− 0.86		− 1.63
	− 0.46		− 1.87		0.15		− 1.44		− 0.01
C_5	2.49	O_5 axial	2.46			H_5 axial	2.52	H_5 equatorial	
	0.00		1.35				− 0.02		
	0.00		− 0.50				0.98		
C_6	3.78								
	− 0.61								
	− 0.51								

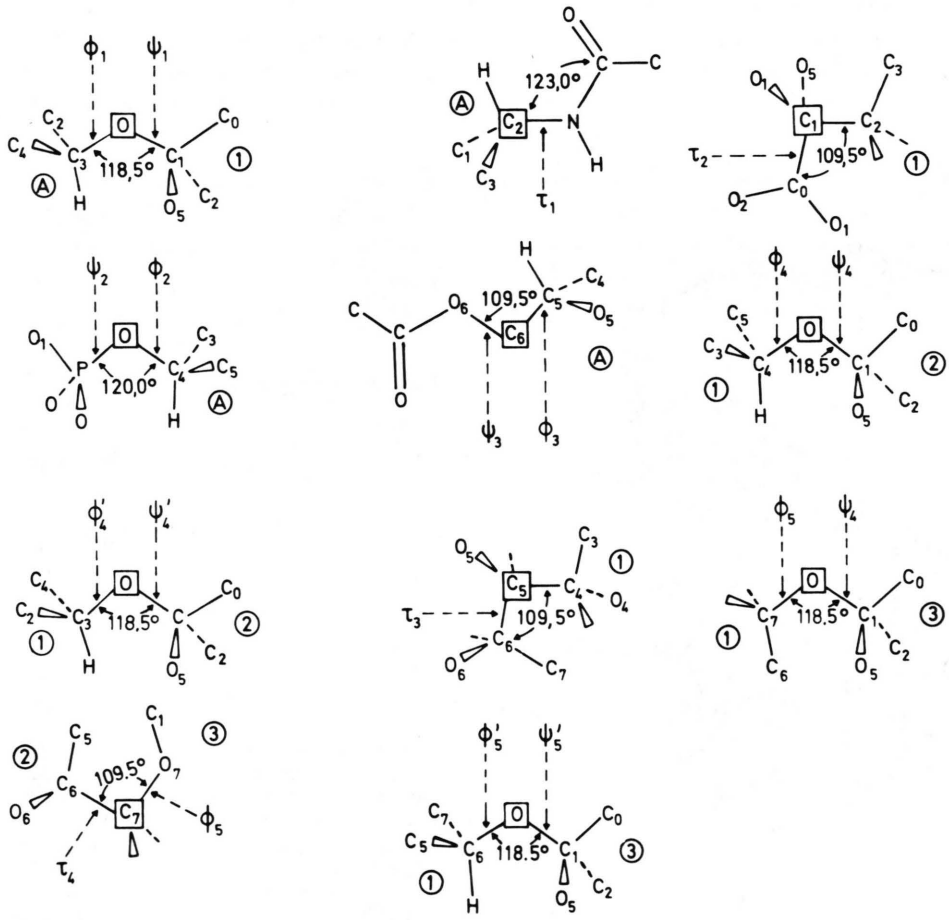


Fig. 3. Zero-positions of the rotation angles. Atoms connected by a solid bar are situated in a common plane Ⓐ = N-acetylglucosamine residue. ① ② ③ = KDO residues as defined in Fig. 2. For the calculations, the origin of the auxiliary coordinate system were transformed into the atoms marked by a square.

origin, the positive X-axis directed towards the C_5-atoms with the C_1-atoms defining the first quadrant of the x/y-plane. The mean deviations of the atomic coordinates are less then 0.05 Å.

The coordinates of the H-atoms were calculated by use of the standard C-C-H bond angle of 109.5° and a C-H bond length of 1.00 Å.

Atomic coordinates of the ester- and N-acetyl-group

Atomic coordinates of 12 ester groups [24–30] and of the N-acetyl group (similar to a peptide bond [31], were taken from literature. The C-O ester bond or the C-N amide bond, respectively, define the positive x-axis with the carbonyl oxygen being in the x/z-plane. Again, the mean deviations of the atomic coordinates are less then 0.05 Å.

Atomic coordinates of the phosphate- and carboxyl-group

Using the distances and angles of the phosphodiester [32–36] and carboxyl groups [37] atomic coordinates for these groups have been calculated.

Conformation of KDO

Periodate oxidation studies on KDO suggest a manno-configuration for the O-atoms of the shugar ring [38]. The absolute configuration of this O-atoms has yet to be determined [38]. In order to select for a reasonable conformation of the KDO residue, we first compared the α- and β-anomers of its C1 and 1C form with the definition of Reeves [39]. According to this definition all axial substituents other then hydrogen introduce an element of instability. The bulky carboxylgroups C_0O_2 and the $C_6(OH)$-C_7 group should therefore occupy equatorial positions. This is only the case in the α-anomer of the C1 form and two possibilities with O_1 and O_3 [40] or O_1 and O_4 [38] in axial positions can be distinguished.

This conformation was consequently chosen for our calculations. Its atomic coordinates are close to those of N-acetylneuraminic acid [41].

Conformation calculations

Specific energy calculations as a tool of determining molecular conformation of polypeptides and oligosaccharides have been used by Scheraga and his coworkers [42, 43]. While they had to consider detailed chemical interaction energies between molecular groups in order to obtain a sequence apecific chain folding it proved to be sufficient for determining the structure of the carbohydrate moiety of the liopolysaccharide, to calculate interaction energies in terms of van der Waals contacts only.

Allowed rotation angles (Fig. 2) of the carbohydrate moiety of the lipopolysaccharide of *Salmonella minnesota* R 595 have been calculated separately ($\tau_1 \ldots \tau_4$) or in pairs (φ_1, $\psi_1 \ldots \varphi_5$, ψ_5) by Ramachandran type calculations [44] in the sequence of presentation. The hydrocarbon chains of the fatty acids were represented by their α-carbon atoms only.

To calculate the atomic coordinates due to the rotation around a chemical bond the axis of rotation was first shifted to the x-axis of a new coordinate system (Fig. 3). The coordinates of the adjacent group of atoms could then be calculated by iterative use of suitable matrix operations describing stepwise rotation around 10 degrees or less (where resolution was to be increased). Rotations are defined positive when counter clockwise. For each conformation, all atomic distances r_{ij} were calculated and inserted into the Kitaigorodskii function for the interaction energy between non-bonded atoms [45].

$$E = 3.5\,(-0.04/z^6 + 8.6\times 10^3 \exp(-13\,z))\,[\text{kcal/mol}]$$

with $z = r_{ij}/r_0$,

where r_0 is the van der Waals distances between the atoms under consideration (Table II).

Distances larger than r_0 are fully allowed, while distances of 0.2 Å below r_0 are termed partially allowed [44]. For $z = 1$ the Kitaigorodskii function has a minimum with $E = -0.072$ kcal/mol. The sum of all Kitaigorodskii energies of one conformation is its interaction energy content. For all rotation angles we calculated both energy diagrams and contour maps of the smallest interatomic distances near the energy minima (energies in kcal/mol, interatomic distances in Å). Sterically allowed regions are enclosed by lines of forbidden interatomic distances.

Table II. Van der Waals distances.

	C	O	N	P	H
C	3.2	2.8	2.9	3.5	2.4
O		2.7	2.7	3.2	2.4
N			2.7	3.2	2.4
H					2.0

Finally, the calculated conformations were used for the construction of a model for the carbohydrate moiety of the lipopolysaccharide by Nicholson molecular models (Labquip, 18 Rosehill Park Estate, Caversham, Reading RG 4 8XE, England).

Results

All rotation angles are defined in Fig. 2. The results are summarized in Fig. 6.

1) Rotation angles between the N-acetylglucosamine residue of lipid A and KDO residue 1 (φ_1, ψ_1) and their dependence on the rotation of the N-acetylgroup (τ_1).

The rotation freedom of φ_1 and ψ_1 is highly restricted to a single conformation which could be determined by use of the coordinates of N-acetylglucosamine residue A and the KDO residue 1 only. Neglecting the protons, the energetically and sterically allowed region is given by values of $\varphi_1 = 20° \pm 10°$ and $\psi_1 = 270° \pm 10°$ (Figs. 4 and 5). Introducing the coordinates of the H-atoms, the sterically allowed region is even smaller. Further consideration of the rotation angle of the N-acetyl group concentrates the sterically allowed region to the angles $\tau_1 = 4°$, $\varphi_1 = 28°$, and $\psi_1 = 274°$.*

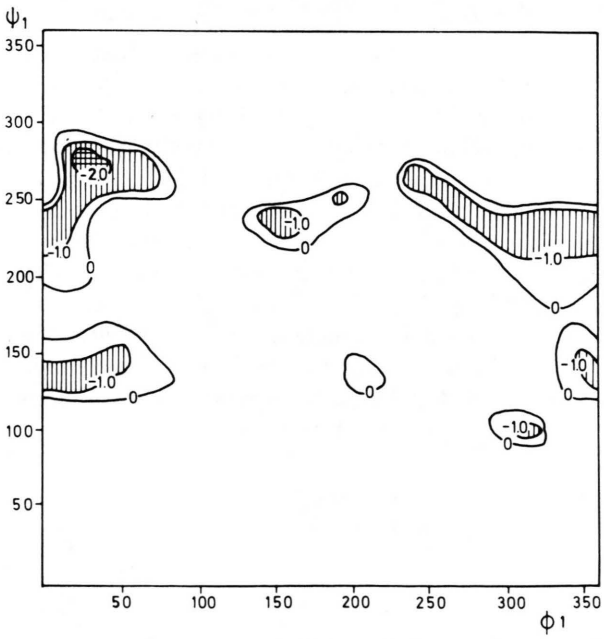


Fig. 4. Energy content for N-acetyl-glucosamine A and KDO 1 as a function of φ_1 and ψ_1.

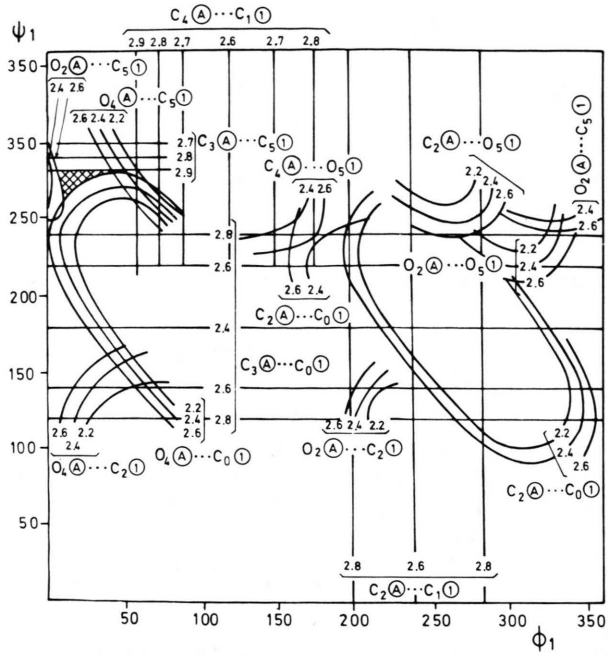


Fig. 5. Smallest distances between N-acetylglucosamine A and KDO 1 dependent on the rotation angles φ_1 and ψ_1.

2) Rotation of the carboxylgroup at C_1 of KDO residue 1 (τ_2)

Using the positions of N-acetylglucosamine residue A and the KDO ring 1 as obtained in the previous section we found allowed conformations for $\tau_2 = 100° \pm 10°$ or $\tau_2 = 280° \pm 10°$. Since the positions of the O-atoms of the carboxylgroup are nearly symmetrical with respect to the $C_1 - C_0$ bond, both values are equivalent.

3) Rotation angles of the phosphate group at C_4 of N-acetylglucosamine residue A (φ_2, ψ_2)

As in the previous sections, the calculation for the rotation angles φ_2 and ψ_2 has been based on the fixed conformation of the N-acetylglucosamine residue A and of KDO ring 1. As a first step we neglected the presence of the phosphate oxygen atoms and restricted the calculations to rotation φ_2. We then found two allowed regions around $\varphi_2 = 190° \pm 5°$ and $\varphi_2 = 330° \pm 5°$. Calculating the rotation around ψ_2 in the next step we obtained three equivalent energy minima for each of the positions

* (The diagrams for all calculations can be obtained from the authors.)

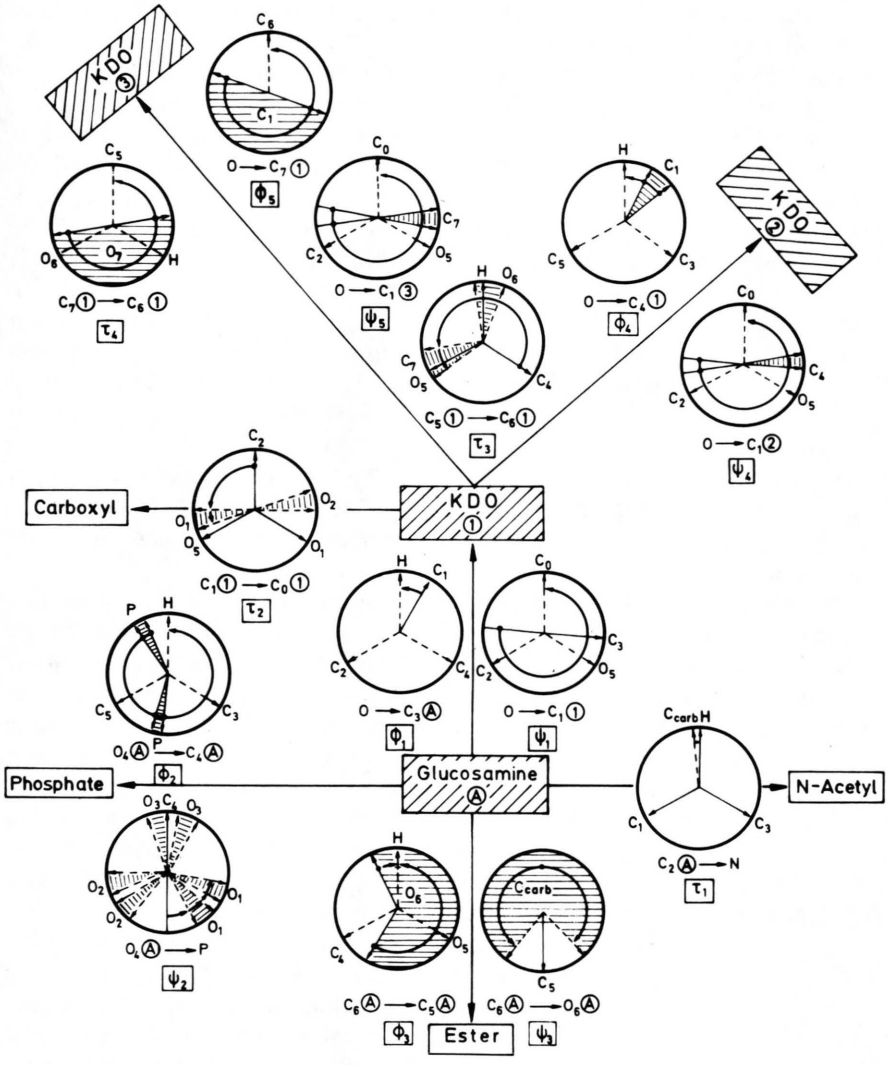


Fig. 6. Scheme of the allowed angular positions in the carbohydrate moiety of the lipopolysaccharide of *Salmonella minnesota* R 595 (see Fig. 1 and 2 for definition). All circles are Newman projections viewing from the center of rotation in the direction of the rotation axis. Directions of viewing and the rotation angle under consideration are given below each circle. The calculated rotation angles are presented by arcs (zero positions see Fig. 3). Shaded areas map ranges of allowed angles.

of φ_2 due to the threefold symmetry of the phosphate group.

$$\varphi_2 = 190° \pm 5° \qquad \psi_2 = 40° \pm 10°$$
$$\varphi_2 = 330° \pm 5° \qquad \psi_2 = 70° \pm 10°$$

4) Rotation angles of the ester group at C_6 of N-acetylglucosamine A (φ_3, ψ_3)

Restrictions for the position of the acetylgroup at C_6 of N-acetylglucosamine due to the presence of the KDO residue 1 and the phosphate group are not severe: a wide range of positions with $-30° \leqq \varphi_3 \leqq 220°$ and $-150° \leqq \psi_3 \leqq +150°$ is allowed.

5) Rotation angles between the KDO residues 1 and 2 (φ_4, ψ_4)

Chemical sequence analysis gives two ways how to link KDO residues 1 and 2 [7].

a) If the KDO residue 2 is linked to O_4 of KDO residue 1 in equatorial position (as assumed in

Fig. 2), an energetically and sterically allowed region is found at $\varphi_4 = 50° \pm 30°$ and $\psi_4 = 275° \pm 15°$. Taking into consideration the coordinates of the H-atoms, too, the sterically allowed region is confined to $\varphi_4 = 35 \pm 15°$ and $\psi_4 = 268 \pm 8°$. All atoms of KDO residue 2 are on one side of the plane through C_1, C_3, C_5 of the N-acetylglucosamine residue A and do therefore not interfere with the hydrophobic region of the fatty acid chains on the other side of this plane. Linkage of KDO residue 2 to O_4 of KDO residue 1 in axial Position is sterically not possible because of close contacts particularly between C_6 of KDO 1 and the carboxylgroup of KDO 2.

b) If the KDO residue 2 was linked to O_3 of KDO residue 1 in axial position only regions around $\varphi'_4 = 30°$, $\psi'_4 = 260°$ would be allowed in the absence of the N-acetylgroup A. If, however, the atomic coordinates of the N-acetylglucosamine residue A are considered as well, forbidden interatomic distances of 2 Å or less are found throughout, and the Kitaigorodskii energies are above zero for all pairs of φ'_4, ψ'_4. Furthermore several atoms of KDO 2 would interfere with the hydrophobic region beyound the plane through C_1, C_3, C_5 of the N-acetylglucosamine residue A. This is also the case, if KDO 2 would be linked to O_3 of KDO 1 in equatorial position.

We therefore conclude that KDO residue 1 should be linked to O_4 of KDO residue 1 in equatorial position.

6) Evaluation of the rotation around $C_5 - C_6$ of KDO residue 1 (τ_3)

Rotation of O_6, C_7, and the proton on C_6 around the $C_5 - C_6$ bond of KDO residue 1 yields two allowed regions: $\tau_3 = 90° \pm 10°$ and $\tau_3 = 250° \pm 50°$.

If in addition KDO residue 2 is considered, we are left with the second region which is now confined to $\tau_3 = 230° \pm 20°$.

7) Rotation angles between the KDO residues 1 and 3 (φ_5, ψ_5, τ_3, τ_4)

Analogous to the KDO 1 – KDO 2 – linkage, sequence analysis suggests two different ways to connect KDO 1 and KDO 3 [7].

a) The KDO residue 3 is linked to the O-atom at C_7 of KDO residue 1 as drawn in Fig. 2. This linkage involves the four rotation angles τ_3, τ_4, φ_5, ψ_5. Allowed regions for the rotation angles φ_5 and ψ_5 have been calculated by use of all KDO 3 atoms and the KDO 1 atoms C_7, its protons, and C_6: $\varphi_5 = 210° \pm 100°$, $\psi_5 = 270° \pm 10°$. Using the values $\psi_5 = 270°$ and $\tau_3 = 230° \pm 20°$ a large range of the angles τ_4 and φ_5 in acceptable: $80° \leqq \tau_4 \leqq 260°$, $110° \leqq \varphi_5 \leqq 290°$.

b) If the KDO residue 3 is linked to the O-atom at C_6 of KDO residue 1, the linkage has three rotation angles termed τ_3, φ'_5, ψ'_5. In this case rotation angles φ'_5 and ψ'_5 were first calculated by use of KDO residue 3 and the KDO 1 atoms C_5, C_6, its protons and C_7. Then ranges around $-60° \leqq \varphi'_5 \leqq 120°$, $240° \leqq \psi'_5 \leqq 280°$ and $\varphi'_5 = 330°$, $\psi'_5 = 110°$ have negative interaction energies and might be allowed.

If the atomic coordinates of N-acetylglucosamine and the KDO residues 1 and 2 are included, we are left with a small region only, which has an energy minimum at $\varphi'_5 = 270° \pm 10°$, $\psi'_5 = 250° \pm 10°$. In this case, however, we find forbidden interatomic distances below 2.8 Å (van der Waals distance 3.2 Å) from C_7 of KDO 1 to C_0 and C_1 of KDO 3. Though each of these distances has a positive Kitaigorodskii energy of about 0.03 kcal/mol, several van der Waals contacts with negative energies cause the energy minimum.

As a consequence, we consider this second kind of linkage from KDO residue 3 to KDO residue 1 as more unprobable than the first one. Note that the linkage of KDO 3 *via* the O atom of C_7 is facilitated by the introduction of one more rotational freedom.

Discussion

The calculation of allowed conformations of the carbohydrate moiety of a lipopolysaccharide of *Salmonella minnesota* R 595 has been based on atomic coordinates derived from literature data for pyranose rings, ester-, N-acetyl-, phosphate-, and carboxylgroups. As for the KDO residues, we based the calculations on the α-anomer of its C 1 form, which has been suggested to be the most probable one. Using the atomic coordinates of its separate components and the knowledge of their chemical links, possible conformations could be calculated for the carbohydrate moiety of the lipopolysaccharide.

At first sterically very unsuitable conformations were exclude by calculating a simple energy function. The succeeding sterical considerations however leave allowed regions in the Ramachandran diagram surrounded by forbidden contacts. These allowed regions are identical with the calculated energy minima. Hydrogen bonds were not considered, since they have no important influence on sterical hindrance and environmental factors can only induce more sterical hindrance.

In Fig. 6 we provide a survey of our results. First we had to consider the rotations around the bonds between glucosamine residue A and KDO residue 1 (φ_1, ψ_1) dependent on the rotation angle of the N-acetylgroup (τ_1). Considerable sterical hindrance restricts, the rotational freedom to just one conformation. Therefore, we could use the coordinates of the complete dissaccharide for all succeeding calculations.

The rotations of the carboxylgroup on KDO residue 1 (τ_2) and of the phosphate group linked to glucosamine A (φ_2, ψ_2) are highly restricted, too, while a large range of angles seems to be allowed for the ester group linked to glucosamine A (φ_3, ψ_3).

Two open problems of sequence analysis could be settled by our sterical calculations.

1) The linkage of KDO residue 2 to O_4 of KDO residue 1 in equatorial position (φ_4, ψ_4) is limited to a narrow range of allowed angles, while a linkage to O_4 and O_3 of KDO residue 1 in axial position is sterically not possible. Furthermore a linkage of KDO 2 to O_3 of KDO 1 in axial or equatorial position would cause interference of atoms from the carbohydrate moiety with the hexagonally packed fatty acid chains [12] of the hydrophobic part of lipopolysaccharide. The positions of φ_4 and ψ_4 restrict the freedom of rotation around the axis C_5-C_6 (τ_3) in KDO residue 1 considerably.

2) Linking KDO residue 3 to the oxygen atom of C_7 of KDO residue 1 introduces four angles of rotation (τ_3, τ_4, φ_5 and ψ_5), two of which (τ_4, φ_5) should be freely rotatable through a wide range. The alternative connection *via* the oxygen atom on C_6 of KDO residue 1, however, would involve a link of three bonds only. We still got one conformation with a negative Kitaigorodskii energy of the sum of interatomic interactions, yet two C - - - C contacts would be forced well below their assigned van der Waals distances.

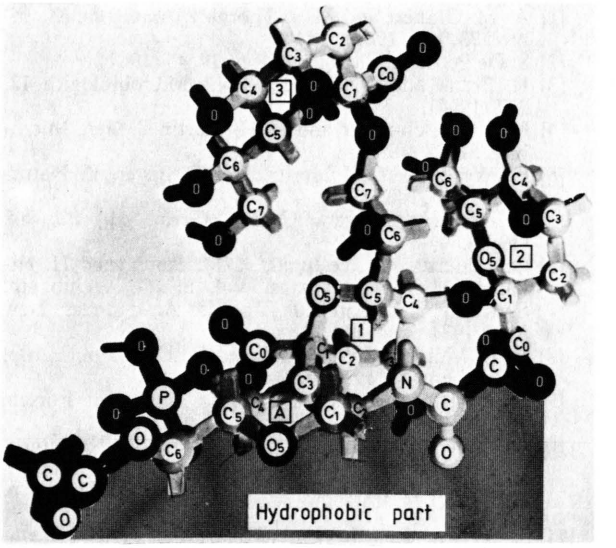


Fig. 7. Three-dimensional atomic model of the carbohydrate moiety of the lipopolysaccharide of *Salmonella minnesota* R 595, consisting of an N-acetylglucosamine residue A, to which a trisaccharide with three KDO residues 1 2 3 is linked (Compare Fig. 2).

Using the data of Fig. 6 an atomic model of the carbohydrate moiety of the lipopolysaccharide from *Salmonella minnesota* R 959 could be built (Fig. 7). The sterically not hindered conformation, demonstrated in this model should have a real probability of existence.

The compact hydrophilic carbohydrate moiety of this model of lipopolysaccharide does not interfere with the hexagonally packed fatty acid residues which form the hydrophobic part of the structure (Fig. 7).

An extension of the calculations to the core- and O-polysaccharides of other serotypes of Salmonella may lead to a sterical explanation of serological reactions

Acknowledgements

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