Possible Orientation of the Fatty Acid Chains in Lipopolysaccharide

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Lipopolysaccharide, Hydrocarbon Chains, Orientation

The fatty acid chains of the lipid A component of lipopolysaccharides are hexagonally packed with a lattice periodicity of 4.1 Å. The smallest subunit of this lattice consists of a disaccharide to which seven fatty acid chains are linked representing the hydrophobic part. Carbohydrates and substituted phosphate residues linked to them form the hydrophilic part of the molecule.

Because of sterical reasons and because of the necessity of a separation of hydrophobic and hydrophilic part, we could derive that the angles of the hydrocarbon chains with the planes of the two sugar residues should be as vertical as possible and the planes of both sugar residues should be approximately coplanar forming an angle of about 180° with one another.

Conformation angles for all the theoretically possible linkages of the disaccaride and of the linkages of the fatty acid residues have been calculated. Between the two-N-acylglucosamine residues theoretically β-1,4, α-1,6 or β-1,6 linkages are possible. The experimentally found β-1,6 linkage has the largest degrees of freedom for its conformation angles.

Introduction

Lipopolysaccharides are constituents of the outer layer of the cell walls of Gram-negative bacteria. The general structure of the lipopolysaccharide of many kinds of bacteria [1] is shown in Fig. 1 a. The O-polysaccharide which is responsible for serological O-specifity points to the outside of the bacterial cell wall. To the O-polysaccharide the group specific core-oligosaccharide is linked and this core is linked, through a trisaccharide consisting of three KDO* residues to a lipid component termed lipid A.

Lipid A of *Salmonella* consists of two β-1,6 linked D-glucosamine residues (Fig. 1 b) each of which carries a phosphate residue to which phosphoethanolamine and a 4-amino-4-deoxy-L-arabinosamine group is linked respectively. The two aminogroups of the disacccharide are substituted with D-3-hydroxymyristic acid residues, while further five fatty acid residues (1 lauryl-, 1 palmityl-, 1 D-3-hydroxymyristic and 1 D-3-myristoxymyristic acid residue) are linked to available hydroxyl-groups (2).

Lipopolysaccharides possessing, due to aggregation a molecular weight of several millions are responsible for pyrogenicity and other symtpoms of Gram negative infections and it has been found [3], that only the lipid A component is responsible for the induction of endotoxic reactions.

* KDO, 2 Keto-3-deoxyoctonate.

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Due to energetic considerations and X-ray diffraction measurements valid for all amphipatic structures [4] also in lipopolysaccharides [5] the hydrophobic fatty acid chains should be parallel to each-other and therefore cause a clear separation of the hydrophobic and the hydrophilic part.

The possibilities for a parallel orientation of the fatty acid chains dependent on different conformations and types of linkages within the dissaccharide unit are described in this publication.

Methods

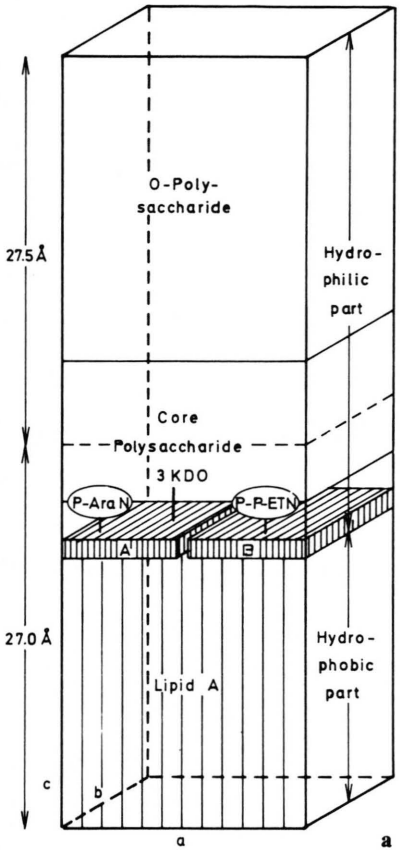
Accurate atomic coordinates for the glucose residues, the ester- and amide linkages have been taken from literature [6]. The coordinates, of the fatty acid chains have been calculated for C-C bond distances of 1.52 Å and bond angles of 112° [7].

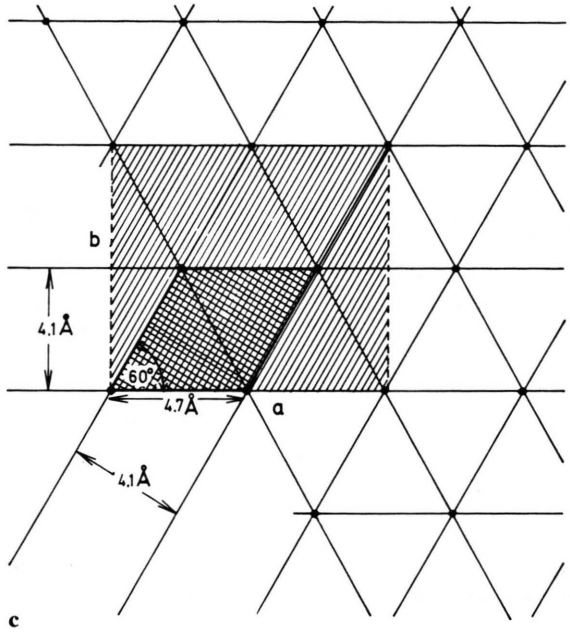
Calculation of the angles between a fatty acid chain linked to a sugar residue and the plane of this residue

Possible angles (α) between the fatty acid chains linked to the amminogroups of glucosamine A and B (Fig. 2) with the planes of these residues made up by the atoms C_1, C_3 and C_5 have been calculated in the following way:

First: The atoms C_1, C_3 and C_5 of the N-acylglucosamine residue have been placed in the x, y plane.

Second: The fatty acid chains are assumed to be in the *all trans* form where the angles τ_1 may assume





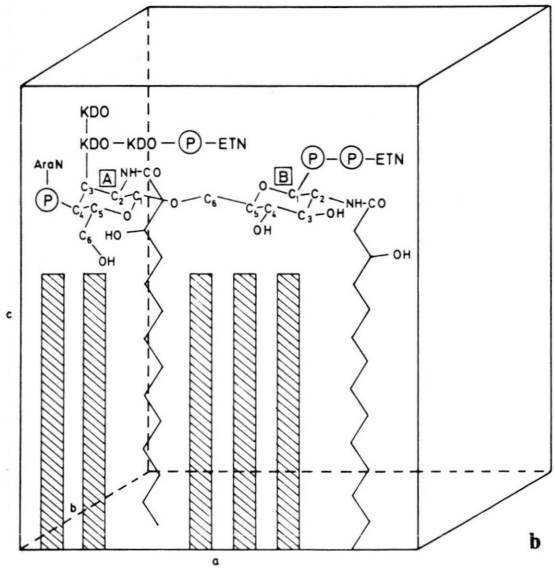


Fig. 1. a) Scheme of the subunit of lipopolysaccharide inserted into an elementary cell ($a \cdot b \cdot c$) $c = 54.5$ Å. b) Formula of the subunit of the Re-mutant of the lippolysaccharide of Salmonella minnesota inserted into an elementary cell ($a \cdot b \cdot c$) $c = 27.0$ Å. c) Two dimensional lattice made up of the fatty acid residues of lipololysaccharide /// orthorhombic elementary cell ($a \cdot b$); ### hexagonal elementary cell; A, B, two glucosamine residues; (P), Phosphate; Ara N, Arabinosamine; EtN, Ethanolamine.

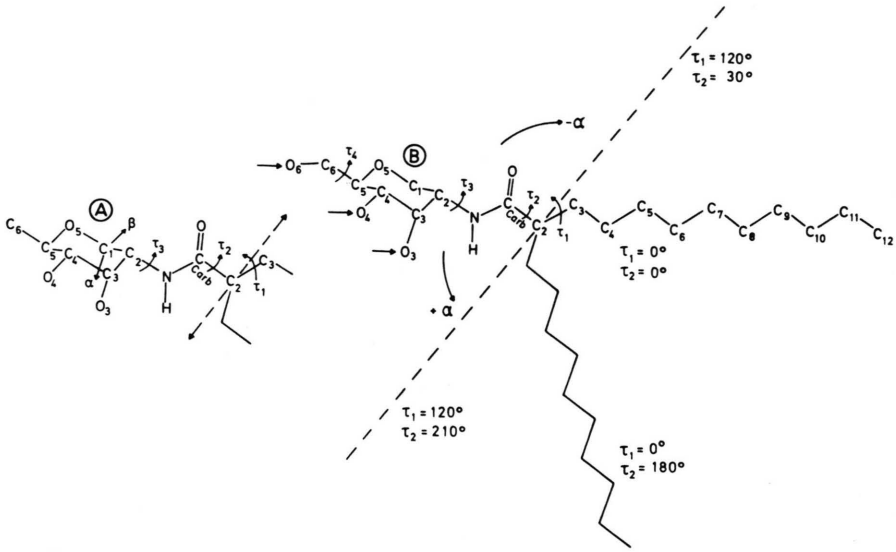


Fig. 2. The two N-acylglucosamine residues A and B of lipid A with the rotation angles τ. α = angle of the N-acyl hydrocarbon chain with the plane of sugar residue B.

two values [8]: $\tau_1 = 0°$ solid zig-zag line in Fig. 2 and $\tau_1 = 120°$ dashed line in Fig. 2.

Sets of coordinates for the C_{12} atoms of the fatty acid chains have been collected for all values of τ_2 from 0° to 360° in steps of 10°. Rotation has been performed clockwise. In the zero position the atoms C_{carb}, C_2 and C_3 are in one plane (Fig. 2).

Third: For the sets of coordinates ($\tau_1 = 0°$, $\tau_1 = 120°$) the angles α between the fatty acid chain and the plane of the sugar residue dependent on τ_2 have been calculated (Fig. 3). Since the plane of the sugar residue is positioned in the x, y plane. The angle α is the direction cosinus between C_2 and C_{12}. The maxima of this angles are also shown in Fig. 2.

Possible conformations for Di-N-acylglucosamine

The following six theoretically possible modes of linkage between the two N-acylglucosamine residues A and B (Fig. 2) have been examined by computer calculations: α-1,3; α-1,4; α-1,6; β-1,3; β-1,4; β-1,6. For this purpose at first the glucosidic oxygen atoms as as centers of rotation have been transfered to the origin of a rectangular coordinate

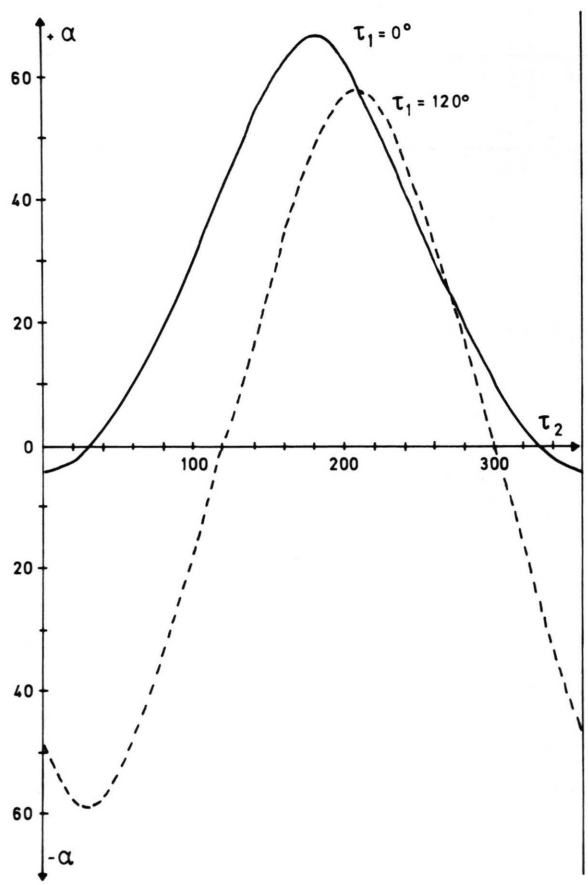


Fig. 3. Angle α between the N-acyl hydrocarbon chain and the plane of the sugar residue to which it is linked, dependent on the rotation angle τ_2 (compare Fig. 2).

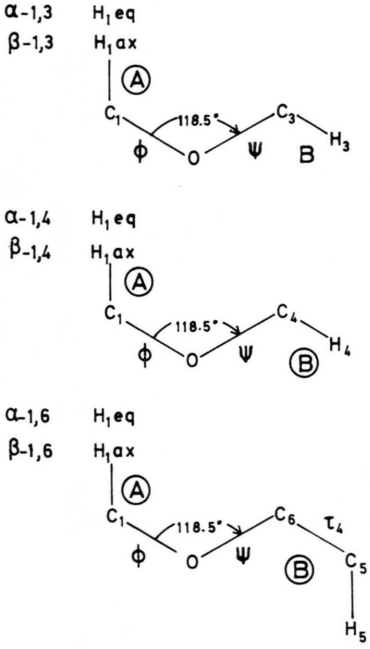


Fig. 4. Zero positions of the rotation angles for the theoretically possible linkages between the N-acylglucosamine residues A and B. All atoms are in the plane of the picture.

system (Fig. 4) and the rotation axes of both sugar residues A and B have been shifted to the x-axis. The atomic coordinates corresponding to different rotation angles φ and ψ have been calculated by iterative use of matrix operations [6, 9]. Rotation was performed in steps of 10° and counter clockwise when viewed from the center of rotation to the C-atom directly linked to it. In order to obtain for sugar residue B the position for all further measurements, for each pair of the rotation angles φ and ψ, residue B has to be turned 118.5°. The zero position of the rotation angles are shown in Fig. 4. For the six theoretically possible modes of crosslinkage between sugar ring A and B (vertical row in Fig. 5), the four combinations of torsion angles τ_1 (horizontal row in Fig. 5) and each pair of the rotation angles φ and ψ calculations on the following subject have been performed:

1) Sterical requirements;
2) angles between the planes of the sugar rings A and B;
3) angles between the fatty acid chains linked to the aminogroups of the sugar rings A and B.

The results of these calculations are summarized in Fig. 5.

1) Sterical requirements

In order to distinguish the sterically allowed conformation from the forbidden ones, interatomic distances between the sugar residues A and B (Fig. 2) have been calculated and compared with the van der Waals table (Table I). For the linkages α-1,3; α-1,4; β-1,3; β-1,4 all atomic coordinates of both sugar residues A and B have been used, while for the linkages α-1,6; β-1,6 which have an additional rotational freedom around the axis C_6-C_5 (τ_4 in Fig. 2 and 4) only the atomic coordinates of sugar ring A and from sugar ring B the coordinates of C_6, C_5 and the two H-atoms at C_6 have been used.

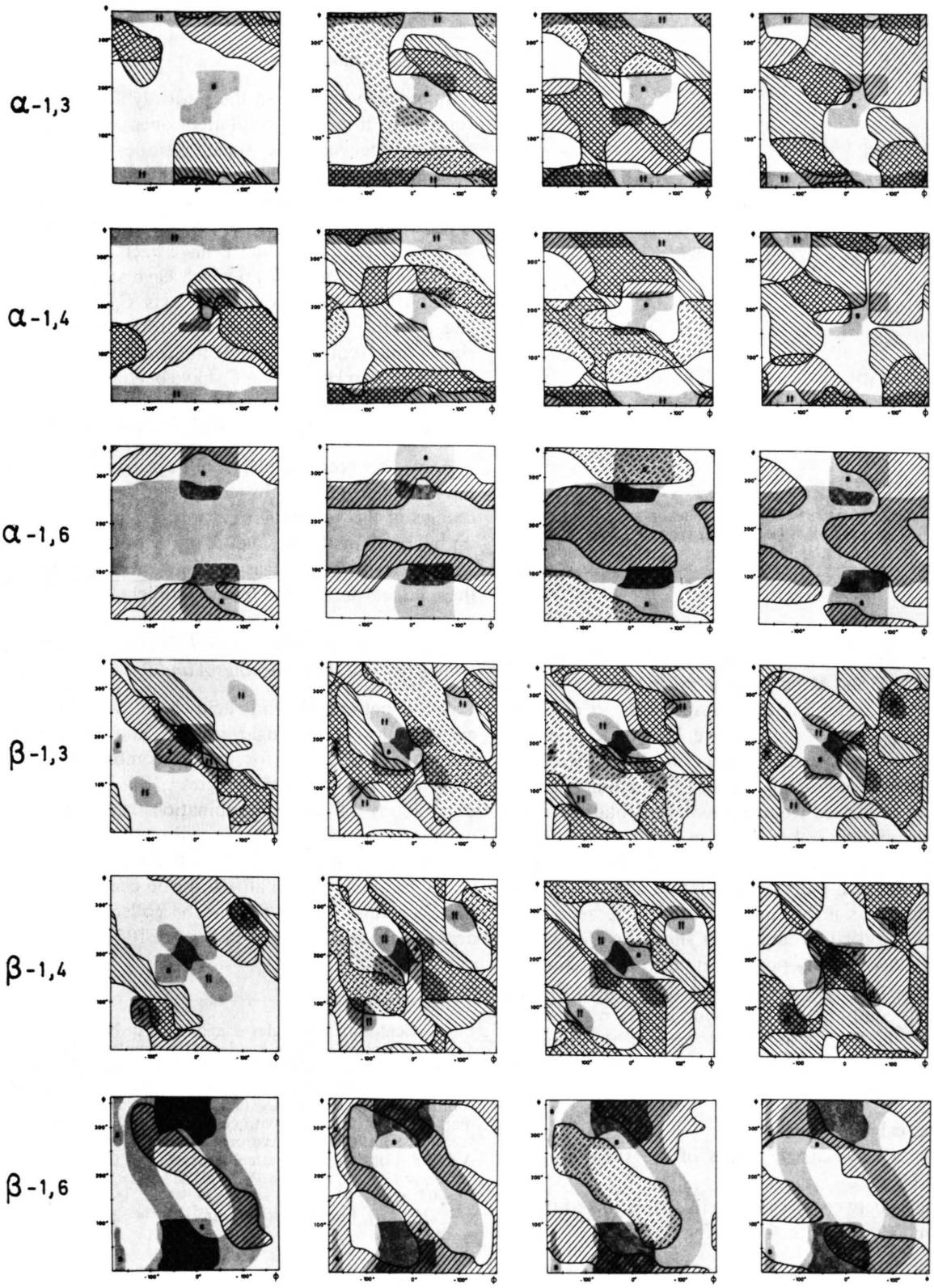
2) Angles between the planes of sugar ring A and B

Angles between the planes of sugar ring A and B have been calculated by comparing the direction cosinus of the vectors normal to these planes made up by the atoms C_1, C_3 and C_5 of the sugar residues. The planes of both sugar residues are coplanar, if these vectors are parallel or antiparallel

3) Angles between the fatty acid chains linked to the aminogroups of the sugar rings A and B

Angles between the fatty acid chains linked to the aminogroups of the sugar rings A and B (Fig. 2) have been calculated for the 24 combinations of crosslinkages with rotation angles τ (Fig. 5). In all φ, ψ positions of these combinations the possible direction cosinus of the fatty acid chains ($C_2 \ldots C_{12}$ in Fig. 2) in sugar ring A and B have been compared. For this purpose at first the 36 coordinates of the C_{12}-atoms (Fig. 2) have to be collected dependent on τ_2 from 0° to 360° in steps of 10°.

Fig. 5. Conformation angles φ and ψ of di-N-acylglucosamine dependent on the mode of crosslinkage (vertical column) and the combination of the τ_1-angles (horizontal column). Grey areas marked with "S" = sterically allowed conformations Grey areas marked with arrows or unmarked = conformations with coplanar sugar rings. Dashed areas for parallel N-acyl hydrocarbon chains in sugar rings A and B. For the three first columns: A, $\tau_1 = 0°$, $\tau_2 = 180°$ ///; $\tau_1 = 120°$, $\tau_2 = 30°$ ///; $\tau_1 = 120°$, $\tau_2 = 210°$ ///. B, $\tau_1 = 0°$, $\tau_2 = 180°$ \\\; $\tau_1 = 120°$, $\tau_2 = 30°$ \\\; $\tau_1 = 120°$, $\tau_2 = 210°$ \\\. For the last column: A, $\tau_1 = 120°$ ///; B, $\tau_1 = 120°$ \\\.


Aτ1 0° 0° 120° 120°
Bτ1 0° 120° 0° 120°
α-1,3
α-1,4
α-1,6
β-1,3
β-1,4
β-1,6

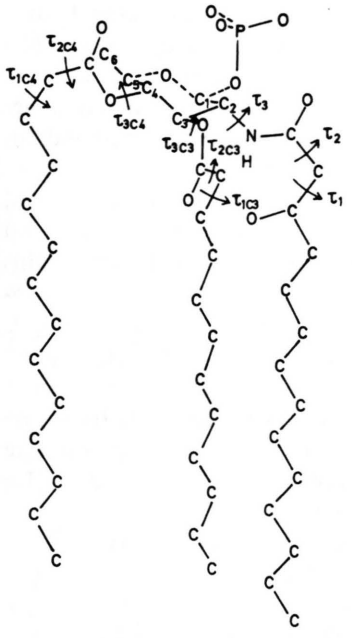


Fig. 6. Possible linkages of fatty acid residues to N-acyl-glucosamine B with rotation angles τ.

Angles between fatty acid residues linked to sugar ring B.

The direction cosinus of the fatty acid chains linked to the aminogroup at C_2 and the ester group at C_3 or C_4 of the sugar residue B have been compared for every combination of rotation angles τ_2, τ_{2C3} and τ_2, τ_{2C4} (Fig. 6) and the four combinations of the τ_1 angles (Fig. 7). Areas of equal direction cosinus (parallel fatty acid chains) are marked as spots in Fig. 7.

Areas for an approximately vertical orientation between the fatty acid chains and the plane of sugar ring B (compare Fig. 3) are marke as dashed columnes (Fig. 7).

Density measurement on foils of lipopolysaccharide of the Re mutant of Salmonella minnesota

Foils of the lipopolysaccharide of the Re-mutant of *Salmonella minnesota* have been prepared by drying a solution in water in a vacuum dessicator over P_2O_5. The foils remained floating when centrifuged in a solution of 30% CsCl ($\varrho = 1{,}286$) for 15 min at 5000 rpm.

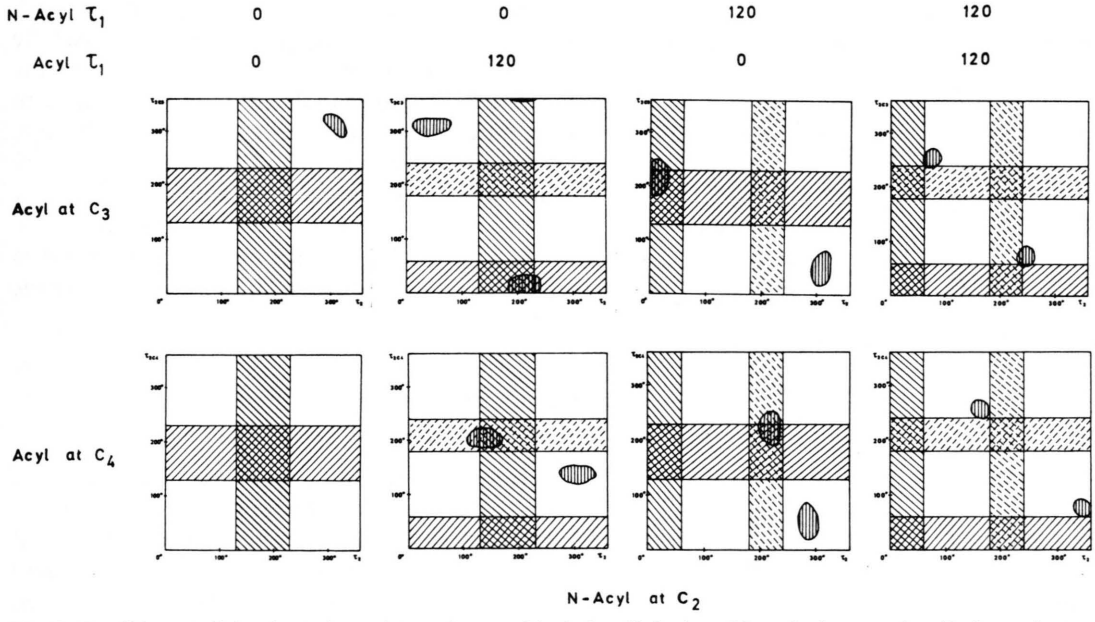


Fig. 7. Possible parallel orientation of two fatty acid chains linked to N-acyl glucosamine B dependent on the four combinations of the τ_1-angles. Horizontal rows τ_2 for the N-acyl residue at C_2, vertical rows τ_2 for the acyl residues at C_3 or C_4. Dashed spots areas of parallelity between the fatty acid chains. Dashed horizontal and vertical columns = Areas of fatty acid chains approaching a vertical position to the plane of sugar ring B (compare Figs. 2 and 3).

Results and Discussion

Comparison between the structure of lipopolysaccharide with that of other amphipatic substances

Similar to other amphipatic substances X-ray diffraction studies provide also for lipopolysaccharides two sorts of spacings usually referred to as long and short spacings [4, 10]. The short spacings are associated with the manner of packing of the hydrocarbon chains while the long spacings are related to integral multiples of the thickness of the bilayer structure in amphipatic substances [4, 10].

For the wild type of the lipopolysaccharide of *Salmonella minnesota* a long spacing of 109 Å has been measured [5] corresponding to a thickness of 54.5 Å for one of the head-head or tail-tail superposed lipopolysaccharide layers. For the Re mutant of *Salmonella minnesota* which contains only three KDO-residues as core a thickness for the monolayer of 27.5 Å has been obtained [5]. For both lipopolysaccharides a short spacing of 4.1 Å has been obtained [5].

Based on experimental results four points determining the amphiphatic structure of lipopolysaccharide can be discussed (Fig. 8).

Point 1: Parallelity between the hydrocarbon chains

The occurrence of one spacing at 4.1 Å in an amphipatic structure should indicate a hexagonal packing [4]. In this arrangement saturated hydrocarbon chains are in the extended *all trans* configuration rotating or in a random orientation about the chain axis [4, 10]. The fatty acid chains thus behave as cylinders whose axes are usually perpendicular to the basal plane of the bilayer.

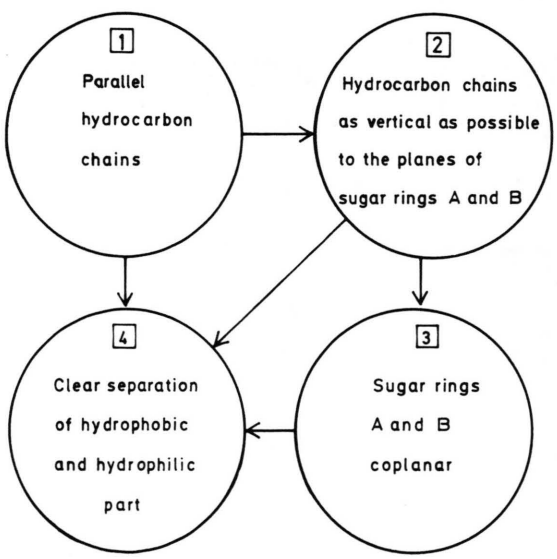


Fig. 8. Diagram of four points determining the amphipatic structure of lipopolysaccharide.

A broad X-ray diffraction band centered around 4.5 Å is correlated with interchain distances and characteristic for disordered hydrocarbon chains [11–13]. A sharp X-ray reflection at 4.1. Å as obtained from dry foils of lipopolysaccharide [5] is caused by lattice distances of 4.1 Å indicating a hexagonal-or centered orthorhombic arrangement of hydrocarbon chains [10] separated 4.7 Å from one another (Fig. 1 c). In this case, the projection of one hydrocarbon chain occupies an area of 19.3 Å^2 [10] on the basal plane (a, b in Fig. 1).

Since seven fatty acid residues directly or indirectly are linked to the disaccharide unit of lipopolysaccharide [2], they occupy an area of $a \cdot b =$ 135 Å^2. For the lipopolysaccharide of the Re-mutant of *Salmonella minnesota* (Fig. 1 b) a density value of $\varrho = 1.286$ was obtained and a molecular weight of 3070 Daltons (Fig. 1 b) calculated. With these values a volume of $V = a \cdot b \cdot c = 3966$ Å^3 can be calculated for the disaccharide subunit of the lipopolysaccharide.

From X-ray diffraction a value of $c = 27$ Å (Fig. 1 b) has been obtained. Therefore it can be calculated, that an area of 147 Å^2 is occupied by the projection of the seven fatty acid residues linked to the disaccharide unit of the lipopolysaccharide. Theoretically the area of $a \cdot b = 147$ Å^2 could be occupied by 7.6 fatty acid residues (19.3 Å^2 per chain). This difference between the calculated value which is slightly higher than the measured one may be due to lattice defects in the lipopolysaccharide layer.

Point 2: Hydrocarbon chains vertical to the planes of the sugar rings

According to point 1 seven parallel fatty acid chains are covalently linked to the disaccharide unit of lipopolysaccharide. Since the maximal length of this disaccharide is about 12 Å, the seven fatty acid residues can due to sterical reasons not be oriented in a linear array. Their spreading over an area of 147 Å^2 can however be arranged if the hydrocarbon chains are oriented approximately vertical to the

planes of the sugar rings A and B (Fig. 1 a) made up by the atoms C_1, C_3 and C_5.

Point 3: Coplanarity of sugar rings A and B

According to point 2 the fatty acid chains should be oriented as vertical as possible to the planes of the sugar rings. Therefore the planes of both sugar rings A and B (Fig. 1 a) should be approximately coplanar.

Point 4: Separation of hydrophobic and hydrophilic part

If the seven parallel fatty acid chains linked to the disaccharide unit of lipopolysaccharide (point 1) are nearly rectangular to the planes of the coplanar sugar rings A and B (point 2 and 3) a clear separation of the hydrophobic and the hydrophilic part of lipopolysaccharide is obtained in agreement with the structure analyses and model considerations of amphipatic structures [7, 8, 10, 11]. The hydrophobic part of lipopolysaccharide contains fatty acid residues with twelve, fourteen and sixteen C-atoms [2] which may extend from 16 to 20 Å in the *c*-direction (Fig. 1 a and b). These values are obtained as thickness of one layer in the hexagonally packed bilayers myristic-, lauric- and palmitic acid [4]. The rest of the 27 Å thick layer of the Re-Mutant of the lipopolysaccharide of *Salmonella minnesota* is occupied by the hydrophilic carbohydrate moiety (~ 7 to 11 Å).

Calculation of possible orientations of the fatty acid residues in lipopolysaccharide and possible conformations of the disaccharide dependent on the mode of crosslinkage

Considering the four points essential for the structure of lipopolysaccharide (Fig. 8) a calculation of the possible orientations of its fatty acid residues has been performed.

Possible conformation angles φ and ψ of the disaccharide dependent on the mode of crosslinkage (Fig. 4) can be derived from Fig. 5.

The vertical column gives the modes of crosslinkage, the horizontal one the combinations of the τ_1-angles of the N-acyl glucosamine rings A and B (Fig. 2). Four parameters have been caluclated and are demonstrated in Fig. 5.

1) Sterical requirements

Sterically allowed are all conformations of the disaccharide with non bonded interatomic distances larger than the distances given in Table 1. These conformations are drawn in Fig. 5 as grey areas marked with "S".

2) Coplanarity between sugar ring A and B (Point 3)

Conformations with coplanar sugar rings are drawn as grey areas marked with parallel arrows for coplanar sugar rings with an angle of $0° \pm 20°$ between their planes (1-fold screw axis) and with antiparallel arrows for coplanar sugar rings with an angle of $180° \pm 20°$ between their planes (2-fold screw axis). Because of the additional free rotation angle τ_4 (Figs. 2 and 4) for α- and β-1,6 linkage in this case both possibilities of coplanarity have not been distinguished.

3) Hydrocarbon chains vertical to the planes of the sugar residues (Point 2)

Similar to peptide bonds [14] the following atoms of N-acylglucosamine should lie in one plane (Fig. 2): C_2 of the sugar ring, N, H at N, C_{carb}, O at C_{carb}, C_2 of the fatty acid residue.

This plane can on sterical reasons only be tilted for $\tau_3 = \pm 40°$ away from the vertical position to the plane of the sugar ring (Fig. 2). The angle α between the fatty acid residue and the plane of the N-acyl-glucosamine residue is a function of three rotation angles τ_1, τ_2 and τ_3 (Fig. 2).

For the staggered *all trans* configuration of the fatty acid chain τ_1 can obtain the values 0°, 120° and 240° where the atomic coordinates corresponding to the last two values can be transferred into one another by rotation around the axis τ_2. Therefore only values of $\tau_1 = 0°$ and $\tau_1 = 120°$ have been considered and for these two values of τ_1 the angles α dependent on τ_2 from 0° to 360° have been calculated. Since the axes of the rotation angles τ_2 and τ_3

Table I. Van der Waals distances.

	C	N	O	H
C	3.0	2.7	2.6	2.2
N		2.5	2.5	2.2
O			2.5	2.2
H				1.8

are nearly parallel to one another and τ_3 can only be rotated for $\pm 40°$, no additional possibilities of α are introduced by τ_3, which therefore has been left at its zero position (Fig. 2). There are three positions for which the fatty acid residue of N-acylglucosamine roughly approximates a vertical position ($\alpha = \pm 90$) to the plane of the sugar ring (Figs. 2 and 3)

$\tau_1 = 120°$ $\tau_2 = 30° \pm 30°$ $-\alpha = 47° - 58°$
$\tau_1 = 120°$ $\tau_2 = 210° \pm 30°$ $+\alpha = 47° - 58°$
$\tau_1 = 0°$ $\tau_2 = 180° \pm 50°$ $+\alpha = 48° - 67°$

4) Parallelity between the N-acyl hydrocarbon chains of sugar ring A and B (point 1)

Conformations where the fatty acid residue vertical to N-acylglucosamine A is parallel to any possible position of the fatty acid residue in N-acetylglucosamine B are marked in Fig. 5. And also the reverse conditions where fatty acid residues vertical to N-acylglucosamine B are parallel to any possible position of the fatty acid residue in A. For conformations where both regions overlap, the two fatty acid chains of sugar ring A and B are parallel (Point 1) and vertical to the plane of the sugar residues (Point 2). And only these conformations are considered to be allowed. Deviations of $\pm 20°$ from parallelity are still considered to be parallel. Because of the additional free rotation angle τ_4 whose axis is nearly coaxial with those of τ_2 and τ_3 (Fig. 2) for α- and β-1,6 linkage only parallelity between the fatty acid residues vertical to N-acylglucosamine A and any possible position of the fatty acid residue in N-acylglucosamine B has been considered. τ_4 values around $+60°$ are preferred [15]. The rotation τ_4 is considered positive when viewed counter clockwise viewed from C_5 to C_6 (Fig. 4). The following conclusion can be drawn from Fig. 5: For α-1,3 and α-1,4 linkage no sterically allowed region is identical with a region of coplanar sugar residues. Therefore these modes of crosslinkage are not allowed. The conditiones for overlapping of sterically allowed regions with regions of coplanar sugar rings and parallel fatty acid chains of sugar ring A and B are only fulfilled for the modes of crosslinkage marked with "+" in Table II.

Table II. Allowed modes of crosslinkage for di-N-acylglucosamine.

A τ_1	0°	0°	120°	120°
B τ_1	0°	120°	0°	120°
α-1,3				
α-1,4				
α-1,6		+	+	+
β-1,3	+			
β-1,4				+
β-1,6	+	+	+	+

Possible parallel orientation of two or three hydrocarbon chains linked to sugar residue A or B

In addition to the hydrocarbon chains of the N-acylgroups considered in the previour chapter, the following OH-groups of sugar ring A and B (Figs. 1 b and 2) may be esterified with fatty acids too:
sugar ring A:

OH-group at C_6

sugar ring B:

OH group at C_4 and C_6 for α- and β-1,3 linkage
OH group at C_3 and C_6 for α- and β-1,4 linkage
OH group at C_3 and C_4 for α- and β-1,6 linkage.

A fatty acid chain linked to C_6 of sugar ring A or B can be oriented parallel to every position of the hydrocarbon chain at the N-acylgroup because of the additional free rotation angle around the bond $C_5 - C_6$. Therefore calculations of the direction cosinus have only been performed for the following combinations of fatty acid residues linked to sugar ring B (Fig. 6):

N-acyl at C_2 plus acyl at C_4 for α- and β-1,3 linkage (Fig. 7),
N-acyl at C_2 plus acyl at C_3 for α- and β-1,4 linkage (Fig. 7),
N-acyl at C_2 plus acyl at C_4 and C_3 for α- and β-1,6 linkage.

Comparisons between the direction cosines of two fatty acid residues at C_2 plus C_4 and C_2 plus C_3 dependent on the angles τ_2 and the four combinations of τ_1 have been performed (Figs. 6 and 7). The combination of angles for both parallelity between these fatty acid chains (dashed spots) and approximately vertical position between each chain and sugar ring B (dashed columnes) are shown in Fig. 7. Only angle combinations, where these two regions overlap provide possible conformations for lipid A. A deviation of $\pm 20°$ from parallelity is still considered as parallel and angles between the fatty acid

Table III. Combination of τ angles in sugar ring B for α- and β-1,3 linkage.

τ_1	0°	0°	0°	120°	120°	120°	120°	120°	120°
τ_2			130° – 170°		195° – 235°	0°			180°
τ_{1c4}	0°	120°	120°	0°	0°	120°	120°	120°	120°
τ_{2c4}			180° – 225°		190° – 230°	60°			240°
α	pos	pos	pos 48° – 66°	neg	pos 54° – 58°	neg – 48°	neg	pos	pos 47°
parallel hydrocarbon chains	–	–	+	–	+	+	–	–	+
angle combinations allowed according to Table II			↑						

Combination of τ angles in sugar ring B for α- and β-1,4 linkage.

τ_1	0°	0°	0°	120°	120°	120°	120°	120°	120°
τ_2		180° – 230°		0° – 35°			60°	230° – 240°	
τ_{1c3}	0°	120°	120°	0°	0°	120°	120°	120°	120°
τ_{2c3}		0° – 35°		180° – 230°			240°	60°	
α	pos	pos 67° – 48°	pos	neg – 48° – 59°	pos	neg	neg – 48°	pos 53° – 47°	pos
parallel hydrocarbon chains	–	+	–	+	–	–	+	+	–
angle combinations allowed according to Table II				↑			↑	↑	

Table IV. Combination of τ angles in sugar ring B for α- and β-1,6 linkage.

τ_1	0°		120°		120°		120°		120°	
τ_2	130° – 170°	180° – 230°	195° – 235°	230° – 240°	0°	0° – 35°	0°	60°	180°	230° – 240°
τ_{1c4}	120°		0°		120°		120°		120°	
τ_{2c4}	180° – 225°		190° – 230°		60°		60°		240°	
τ_{1c3}		120°		120°		0°		120°		120°
τ_{2c3}		0° – 35°		60°		180° – 230°		240°		60°
α		pos		pos		neg		neg		pos
parallel hydrocarbon chains		+		+		+		–		–

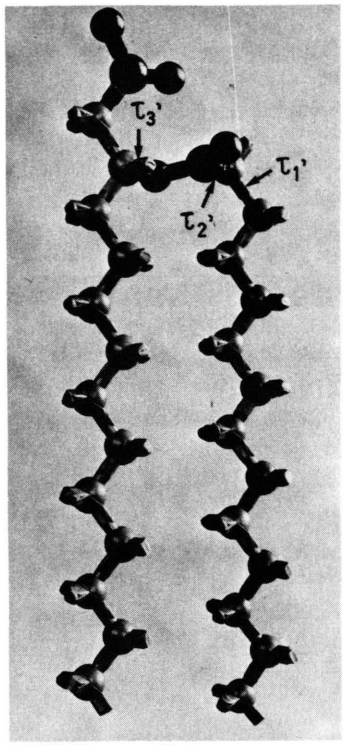


Fig. 9. Myristomyristic acid residue with parallel fatty acid chains.

chains and the plane of sugar ring B from 47 to 67° are considered as best obtainable vertical angles (Fig. 3).

Four possibilities of parallelity (Table III) have been obtaind both for the pair of fatty acid residues linked to C_2 plus C_4 for α- or β-1,3 linkage and to C_2 plus C_3 for α- or β-1,4 linkage. According to Tab. II however in sugar ring B only a τ_1 value of 0° is possible for β-1,3 linkage and a τ_1 value of 120° for β-1,4 linkage. Therefore in sugar ring B only one combination of angles is possible for β-1,3 linkage and three combinations for β-1,4 linkage (arrows in Table III). In the case of α- or β-1,6 linkage three fatty acid residues can be linked to C_2, C_3 and C_4 of sugar ring B. If all the values of Table III are rearranged in Table IV, one can see, that three combinations of angles are possible.

Fatty acid chains linked to the OH-groups of 3-D-hydroxymyristic acid residues

If a fatty acid chain is linked to the OH-group of a 3-D-hydroxymyristic acid residue in the *all trans* configuration, it can only be parallel oriented to this chain if it assumes the following angles of τ' (Fig. 9): $\tau_1' = 120°$, $\tau_2' = -90° \pm 40°$, $\tau_3' = 0° \pm 40°$. Because of the parallelity of the axes of τ_2' and τ_3' these angles are dependent on one another.

Separation of hydrophobic and hydrophilic part of lipopolysaccharide

Beside the seven hydrophobic fatty acid residues three hydrophilic residues are linked to the disaccharide unit of lipid A (Fig. 1 a and b). Newman projections of the sterical possibilities of these linkages are shown in Fig. 10.

Hydrophilic residues linked to sugar ring A

A trisaccharide consisting of three KDO-residues and a phosphate group with an arabinosamine residue are linked to sugar ring A of the Re-mutant of lipopolysaccharide. In order to separate hydrophobic and hydrophilic residues they have to point in opposite direction of the fatty acid chains away from the plane of the sugar residue. The trisaccha-

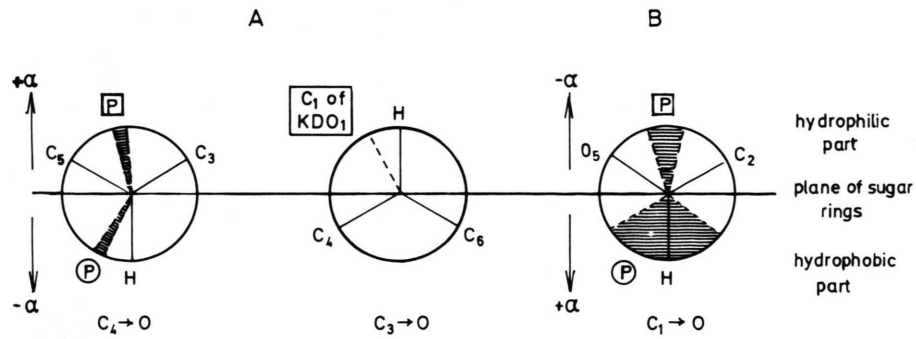


Fig. 10. Newman projection for the hydrophilic residues linked to the di-N-acylglucosamine of lipopolysaccharide.

Table V. Combination of rotation angles for possible modes of crosslinkage.

	A: $\tau_1 = 120°$		A: $\tau_1 = 120°$		
	B: $\tau_1 = 0°$		B: $\tau_1 = 120°$		
α- and β-1,6 linkage	$\tau_2 = 130° - 230°$	α- and β-1,6 linkage	$\tau_2 = 195° - 235°$	β-1,4 linkage	$\tau_2 = 230° - 240°$
	$\tau_{1c4} = 120°$		$\tau_{1c4} = 0°$		$\tau_{1c3} = 120°$
	$\tau_{2c4} = 180° - 225°$		$\tau_{2c4} = 190° - 230°$		$\tau_{2c3} = 60°$
	$\tau_{1c3} = 120°$		$\tau_{1c3} = 120°$		
	$\tau_{2c3} = 0° - 35°$		$\tau_{2c3} = 60°$		

ride at C_3 can on sterical reasons [6] only point in the positive direction (+α Fig. 10). This direction is also possible for one of the sterically allowed linkages of the phosphate residue at C_4 [6] (Fig. 9), while the other possibility would place the phosphate group in the plane of sugar ring A where it would interfere with the packing of the fatty acid residues. The N-acyl residue can therefore only point in the negative direction of sugar ring A (Figs. 3 and 10) where only one combination of angles is possible: $\tau_1 = 120°$, $\tau_2 = 30°$ (Fig. 3).

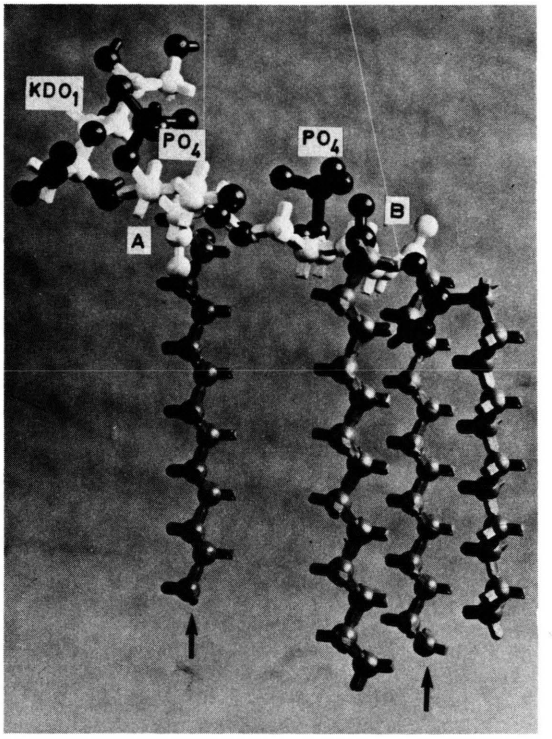


Fig. 11. Model of lipid A with β-1,6 linked N-Acylglucosamine residues A and B. Two phsophate (PO_4) one sugar (KDO_1) and four fatty acid residues are linked to the disaccharide. The two N-acyl fatty acid residues are marked with arrows. The two other fatty acid residues are linked to O_3 and O_4 of sugar residue B.

Hydrophilic residue linked to sugar ring B

The pyrophosphoethanolamine residue linked to C_1 of sugar ring B has again two sterically allowed possibilities of linkage (Fig. 10). One in the negative direction of the sugar plane (− α) and one in the positive direction (+ α) where it would interfere with the packing of the fatty acid residues. The N-acyl residue can therefore only point in the positive direction of sugar ring B (Figs. 2, 3, 10) where two combinations of angles exist: $\tau_1 = 0°$, $\tau_2 = 180°$ and $\tau_1 = 120°$, $\tau_2 = 210°$ (Fig. 3).

Conclusion

Because of the separation of the hydrophobic and hydrophilic portion in lipopolysaccharide all fatty acid residues linked to sugar ring A have to point in the negative direction and all fatty acid residues linked to sugar ring B in the positive direction of the sugar plane. Both planes should therefore form an angle of about 180° (2 fold screw axis) between one another and only the angle combination $\tau_1 = 120°$, $\tau_2 = 30°$ in sugar ring A with $\tau_1 = 0°$, $\tau_2 = 180°$ or $\tau_1 = 120°$, $\tau_2 = 210°$ in sugar ring B are allowed (framed in Table II). These are the crosslinkages α- and β-1,6 and β-1,4 with the angle combinations shown in Table V. The experimentally found β-1,6 linkage has the largest degree of freedom for its conformation angles.

Fig. 11 shows a model of lipid A with β-1,6 linked N-acyl-D-glucosamine residues A and B. The hydrophilic phosphate groups and the sugar residue KDO_1 point in one direction of the plane made up by the coplanar sugar residues, while the hydrophobic fatty acid residues point in the other direction.

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