Possible Models of Murein and Their Fourier Transforms

Helmut Formanek

Botanisches Institut der Universität München, Menzinger Straße 67, D-8000 München 19

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Murein, Models, Fourier Transforms

Murein, the rigid layer of the cell walls of almost all bacteria can be regarded as derivative of chitin. Within the sterically allowed region its polysaccharide chain can perform conformations with two-to threefold screw axes. Two dimensional Fourier transforms calculated from different possible conformations have been compared with data of density measurements, X-ray and electron diffraction. The Fourier transform of murein with a chitin-like conformation of the polysaccharide chain and an elementary cell of $4.5 \times 10.4 \times 21.5$ Å^3 provides the best agreement with the experimental results.

Introduction

Murein or peptidoglycan is the rigid layer of the cell walls of almost all bacteria. Chemically it can be regarded as a derivative of chitin (Fig. 1). The latter is a polymer consisting of β-1,4-linked N-acetyl glucosamine residues. As distinct from chitin, in murein the OH-groups at C-3 of every second sugar residue forms an ether link to a D-lactyl residue. This derivative of N-acetylglucosamine is called N-acetylmuramic acid. Tetrapeptides consisting of alternating L- and D-amino acid residues are bound to the carboxylgroups of the muramic acid residues. The peptides may be crosslinked between the ω-amino group of a diamino acid residue and the carboxylgroup of a D-alanine residue of an adjacent peptidoglycan strand. In this way murein forms a tight network around the bacterial cell [1] (Fig. 2).

The cell walls of Gram negative bacteria have a murein layer which is about 20 Å thick [2–4]. In addition they contain proteins, lipoproteins, lipids and lipopolysaccharides. The probably multilayered cell walls of Gram positive bacteria are about 300 Å thick [5] and consist of murein combined with polysaccharide teichoic- and teichuronic acid.

X-ray diffraction on murein foils of both Gram positive and Gram negative bacteria showed diffuse Debye-Scherrer rings corresponding to distances of about 4.5 and 10 Å [6–10]. Density gradient [6, 9] and infrared measurements [13] on both mureins are also similar. Low dose electron diffraction on thick murein layers of gram positive bacteria provide a diffuse Debye-Scherrer ring at 4.5 Å [11,12] while lattice lines 4.5 Å distant from one another could be visualized with high resolution electron microscopy on the thin murein layer of a Gram negative bacterium [14]. Possible three dimensional models have been, proposed, by several authors [6, 8–10, 15–17]. The sterical requirements of the polysaccharide chain of murein can be expressed with calculations performed first by Ramachandran [18] on cellulose.

In our work calculations have been performed on the sterical requirements and helical parameters of the polysaccharide chain of murein. Fourier transforms have been calculated with these helical parameters and the atomic coordinates of different sterically allowed conformations of murein. An explanation of different models of murein has been tried by comparing these calculated Fourier transforms with the results of physical measurements.

Methods

Atomic coordinates

To yield accurate atomic coordinates, crystallographic data of hexoses have been transformed to a common rectangular coordinate system and averaged [19]. The known atomic coordinates of the carbohydrate residues may be placed in rectangular boxes ($a = 1.5$ Å, $b = 3.5$ Å, $c = 6.0$ Å) (Fig. 1). The atomic coordinates of the lactyl residues and the peptide chains are assumed to be randomly distributed in other rectangular boxes ($a = 1.5$ Å, $b = 6.0$ Å, $c = 15$ Å) (Fig. 1).

Since the atomic coordinates of the carbohydrate residues are restricted to a narrow band of $a = 1.5$ Å and the van der Waals distances between C, O and N atoms are about 3 Å, the carbohydrate residues

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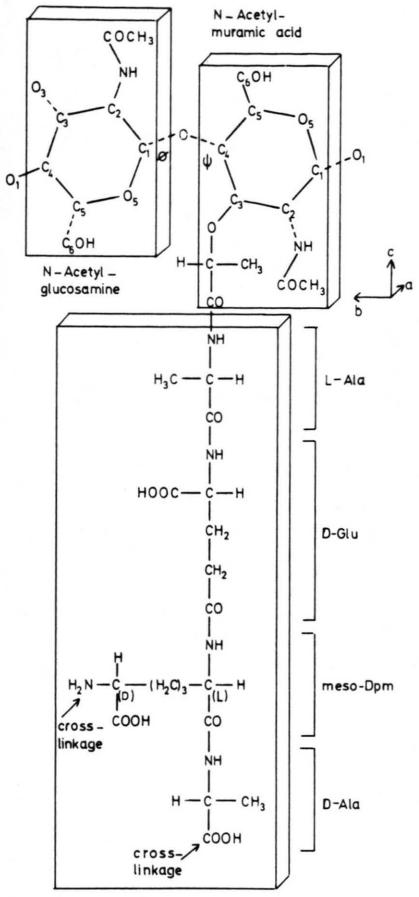

Fig. 1. Formula of a disaccharide peptide subunit of murein. The atomic coordinates of the peptide and the sugar residues are placed in rectangular boxes.

can be packed with a narrow periodicity of 4.5 Å in the a-direction (Fig. 1). Therefore the dimension $a = 1.5$ Å has also been chosen for the box with the coordinates of the peptide. A restriction of its further dimension to $b = 6.0$ Å should enable a packing of

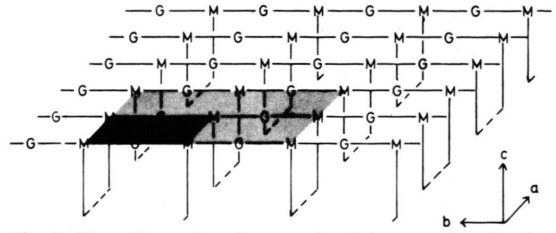

Fig. 2. Two dimensional network of the murein of a Gram negative bacterium. The solid vertical lines symbolize peptides, the dashed lines crosslinkage. Grey area face centered elementary cell. Dark area primitive elementary cell. M = N-acetylmuramic acid; N = N-acetylglucosamine.

about 10 Å in this direction. The dimension $c = 15$ Å has been assumed for the peptide box since the thickness of the Gram negative murein layer is about 21 Å [7] and $c = 6$ Å are occupied by the coordinates of the carbohydrate residue. These still arbitary assumptions for the dimensions of the "black box" with the coordinates of the peptide chain does, however, not influence the calculation on periodic arrangements within the murein layer.

Calculation of possible conformations of murein

Atomic coordinates corresponding to different rotation angles Φ and Ψ have been calculated with a computer program using matrix operations [18]. Rotation was performed in steps of 10 degrees. For each pair of conformation angels Φ, Ψ all interatomic distances were calculated, in order to obtain contour maps of interatomic distances. Sterically allowed regions are enclosed by lines of forbidden contacts (Fig. 3).

These are distances smaller than the sum of the van der Waals contacts between the atoms under consideration. In the zero position $\Phi = 0$, $\Psi = 0$ the glucosidic oxygen and the linked atoms C_1, H_1 and C_4, H_4 are in one plane. H_1 and H_4 point in opposite directions. Since the positions of the H-atoms are not determined as accurate as that of the other atoms, more accuracy for the zero position could be obtained by replacing the H-atoms by vectors vertical to the plane made up by the atoms C_1, C_3 and C_5 of the hexose residues. Rotations are defined positive, when counter clockwise viewing from the glucosidic oxygen to C_1 or C_4. Postion $\Phi = 0$, $\Psi = 180$ is demonstrated in Fig. 1.

Calculation of helical parameters and hydrogen bonds

The polysaccharide chain will assume a regular conformation for a regular sequence of the angels Φ and Ψ. For the sequence $\Phi_2 = \Phi_1$, $\Psi_2 = \Psi_1$; $\Phi_3 = \Phi_1$, $\Psi_3 = \Psi_1$ the helical parameters n (= number of residues per turn) and h (= height of a residue along the helical axis) have been calculated for all pairs of angles obtained by stepwise rotation of 10 degrees using matrix operations (Fig. 4 and 5) [20, 21].

For all pairs of angles also the distances $O_3 \ldots O_5$ and the angles C_3, O_3, O_5 of hydrogen bonds possible between every second sugar residue (Fig. 1) have been calculated dependent on the glucosidic angle (Fig. 6).

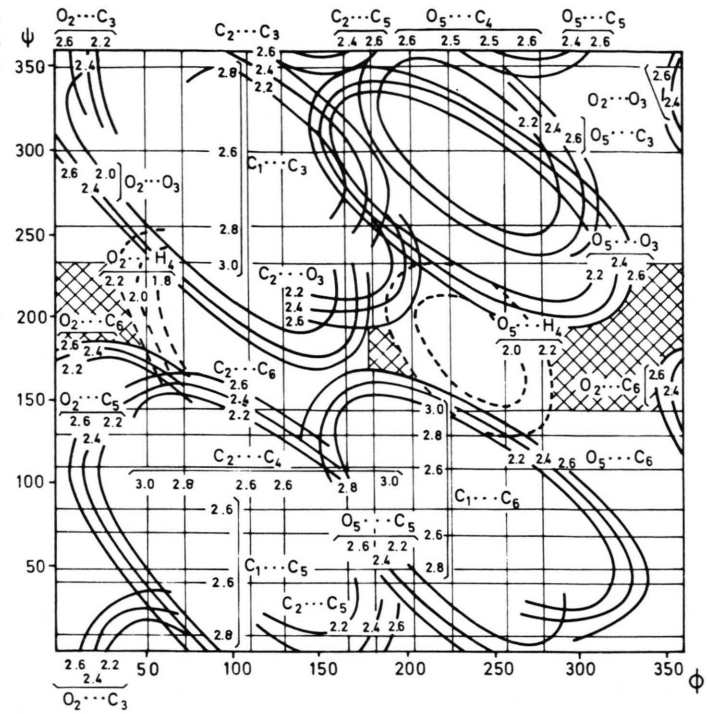

Fig. 3. Distances between two β-1,4 linked glucose residues dependent on the rotation angles Φ and Ψ. The marked areas are sterically allowed.

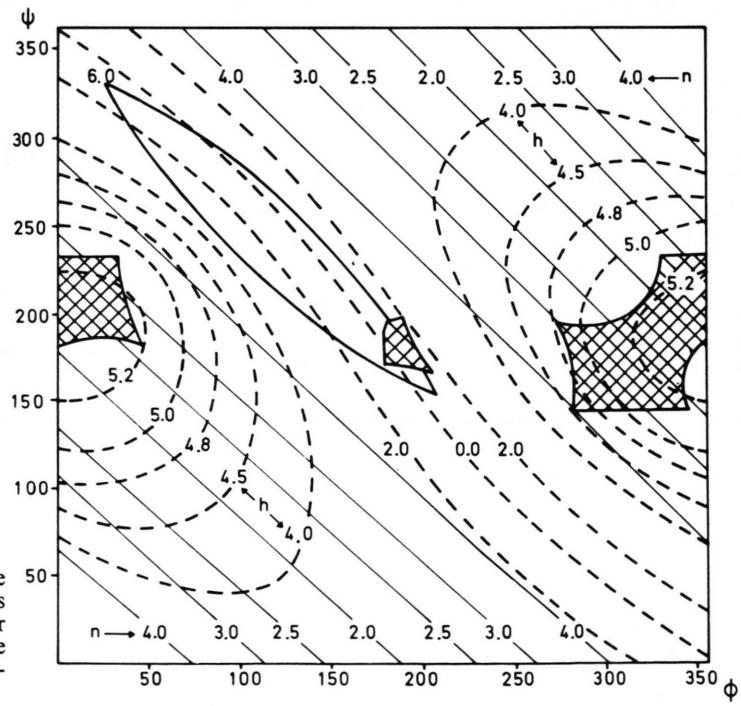

Fig. 4. Helical parameters of a polysaccharide consisting of β-1,4 linked glucose residues (cellulose, chitin). n = number of residues per turn (———); h = height of a residue along the helix axis (– – – –). The marked areas are sterically allowed.

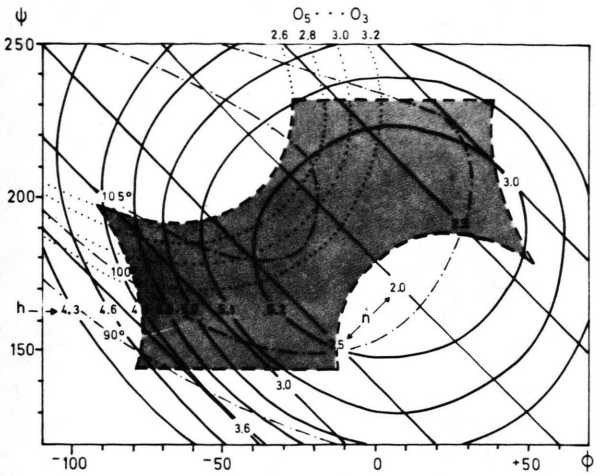

Fig. 5. Sterically allowed (dark) region of the rotation angles Φ and Ψ for β 1,4 linked glucose residues. Glucosidic angle 118.5°. Helical parameters: n = number of residues per turn; h = height of a residue along the helix axis. Length of hydrogen bond $O_5 \ldots\ldots O_3$ (. . . . .) Angle $C_3O_3O_5$ at the hydrogen bond (– · – · –)

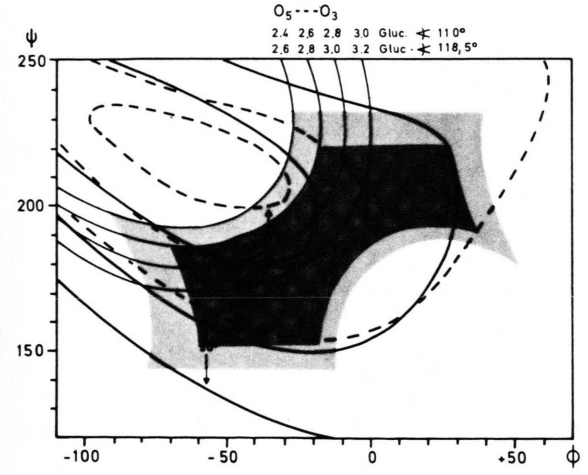

Fig. 6. Sterically allowed region of the rotation angles Φ and Ψ for β-1,4 linked glucose residues. Glucosidic angle 118.5° grey area; glucosidic angle 110° dark grey area. Angle $C_3O_3O_5$ at the hydrogen bond. Glucosidic angle 118.5° (——) glucosidic angle 110° (– – – –)

The sequence $\Phi_2 = -\Phi_1$, $\Psi_2 = -\Psi_1$; $\Phi_3 = +\Phi_1$, $\Psi_3 = +\Psi_1$ is on sterical reasons only possible for a few combinations of angles. This sequence, like all sequences of angles Φ, Ψ lying symmetrical on alternate positions at the right and left side of the axis $n = 2$ (Fig. 5), provides identically repeating disaccharide units where sugar residues 1 and 3, 2 and 4 etc. have identical positions on a screw axis $n = 1$.

Fourier transforms

Because of the minute thickness of the murein layer of Gram-negative bacteria (21.5 Å) [7] only two-dimensional Fourier transforms of monolayers of murein in the $a \cdot b$ plane of Figs. 1 and 2 have been calculated. Calculations have been performed with the atomic coordinates of different sterically possible conformations of murein, their repeating periodicities in direction b (Table I) and different packing periodicities in direction a of Figs. 1 and 2.

Calculations have been performed both with the carbohydrate moiety and the carbohydrate moiety substituted with peptide chains on every second sugar residue and both with and without atomic coordinates of crosslinking residues.

Results

Sterical requirements of the carbohydrate chain of murein

Sterically allowed rotation angles Φ, Ψ (Fig. 1) of the carbohydrate chain of murein have been determined by Ramachandran type calculations [18]. Since sterical hindrance due to the N-acetyl- and lactylgroups can be overcome by rotations around the bonds C_2–N or C_3–O (Fig. 1), calculations have been performed for β-1,4-linked glucose residues only. In the crystalline state the positions of the N-acetyl and the lactylgroup have already been determined by X-ray structure analysis of N-acetylglucosamine [22] and N-acetylmuramic acid [23].

The sterically allowed region is surrounded by lines of forbidden contacts and is limited to

Fig. 7. Calculated two-dimensional Fourier-Transforms of peptidoglycan chains of different conformations packed with periodicities of 10 Å. Screw axis of the polysaccharide chain:
a) 2.0 fold b) 2.5 fold c) 2.66 fold d) 3.0 fold. The numbers give intensities in percent of the l 0 reflex (packing periodicity). First numbers (in dark rows): polysaccharide chains alone; second numbers: polysaccharide chains with peptide chains linked to every second sugar residue.

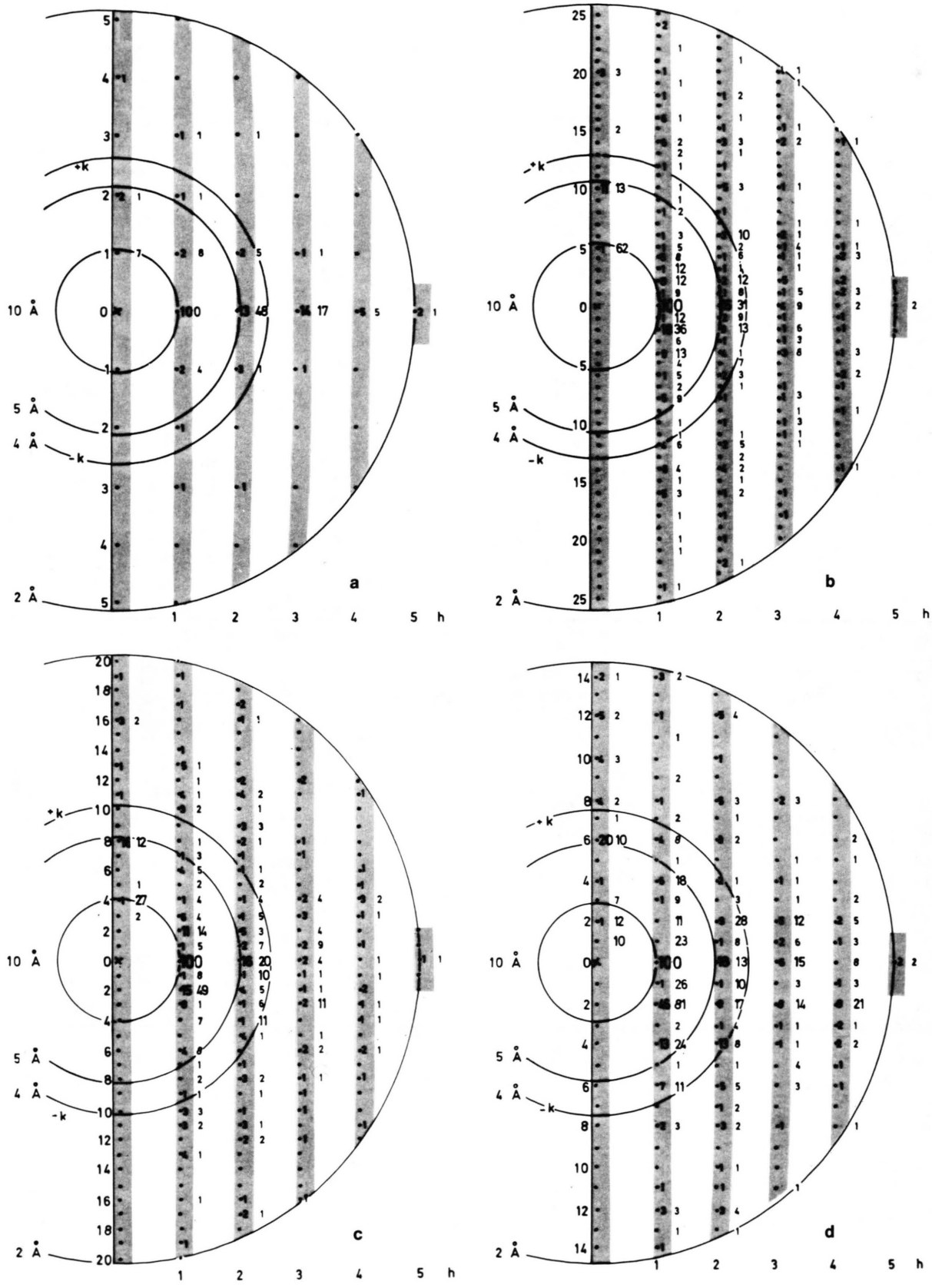
10 Å
5 Å
4 Å
2 Å
+k
-k
a
b
c
d
1 2 3 4 5 h

Table I. Parameters of sterically possible conformations of murein.

Number of residues per turn		2.0	2.5	2.66	3.0
φ, ψ values corresponding to structures for which the Fourier transform has been calculated	φ	330°	310°	300°	280°
	ψ	210°	190°	190°	190°
Height of the disaccharide peptide periodicity along the helical axis		10.38 Å	10.28 Å	10.14 Å	9.78 Å
Reflex corresponding to this height		0 1	0 5	0 4	0 3
Repeating periodicity in the peptidoglycan chain (= length of elementary cell in the direction of the helix axis; direction b in Fig. 1 and 2)		10.38 Å	51.40 Å	40.56 Å	29.34 Å
Number of disaccharide units per repeating periodicity, identical with the number of peptide residues per turn		1	5	4	3

$-80° < \Phi < +50°$ and $140° < \Psi < 230°$ (Figs. 3 and 5) if the glucosidic angle is 118.5°. Smaller glucosidic angles (110°) cause even smaller allowed regions ($-60° < \Phi < +40°$; $150° < \Psi < 220°$) (Fig. 6). For the glucosidic angle 118.5° the region around $\Phi = 200°$, $\Psi = 180°$ (Fig. 3) is sterically not hindered in the case of a disaccharide. The rise along the helix axis per repeating unit is, however, nearly zero so that a polysaccharide chain is sterically hindered. Within the really allowed region around $\Phi = -20° \pm 60°$; $\Psi = 180° \pm 50°$ (Fig. 5) the polysaccharide chain forms two to threefold screw axes with rises of 4.8 to 5.3 Å per sugar residue along the helix axis (Figs. 4 and 5). Within this region the polysaccharide chain may be stabilized by interchain hydrogen bonds between O_3 and O_5 (Fig. 1) if the distances between these atoms are between 2.6 and 3.0 Å and the angle C_3, O_3, O_5 is about 100° or larger (Figs. 5 and 6). Lower values for these angles result, if the glucosidic angle decreases (Fig. 6). Because of the lactyl residues in murein, these hydrogen bonds can only be formed at every second glucosidic linkage.

Fourier Transform

Table I shows the helical parameters of different conformations of murein. The two dimensional Fourier transforms of these conformations are given in Fig. 7 with the assumption of a packing periodicity of 10 Å for the peptidoglycan chains. The 1 0-reflex corresponding to the packing periodicity (10 Å) is the most intense one in all these calculated diagrams, and its higher orders occur still with considerable intensities. Reflexes have been calculated both for the unsubstituted polysaccharide chains and for the polysaccharide chains substituted with peptide chains on every second sugar residue. The periodicity of these disaccharide peptide chains causes the 0 k reflexes in the region of 10 Å (Table I).

A much closer packing periodicity of about 4.5 Å can be obtained in the case of a twofold screw axis as in chitin [24]. Again the 1 0-reflex for the packing periodicity (4.5 Å) is the strongest one (Fig. 8). Only about 10% or less of its intensity have been calculated for the 2 0 reflex at 2.25 Å and the 1 1 and 1 $\bar{1}$ reflexes at 4.13 Å. A stronger 0 1-reflex with about 20% of the intensity of the 1 0-reflex is caused by the repeating periodicity of the peptide chains. The intensities caused by the peptide chains can decrease, if their coordinates are rotated around the *a*- or *b*-axis (Fig. 1) thus approaching a continous distribution of the projections of their coordinates in the *a*, *b* plane.

Crosslinking of the peptidoglycan chains may cause a face centered elementary cell (Fig. 2) and for a chitin like packing of the polysaccharide chains an elementary cell of 9.0×20.8 Å would result. In addition to the reflexes of the primitive cell mainly two

additonal reflexes at 8.25 Å occure (Fig. 8). They are very pronounced, if the coordinates of the crosslinking diamino acids are diagonally oriented in the elementary cell.

Till now all two dimensional Fourier calculations have been performed for rectangular elementary cells $a \cdot b \cdot c$ (Figs. 1 and 2) with different numbers of residues per turn (n), different height (h) of one residue along the helix axis (b in Figs. 1 and 2) and different packing periodicities (a in Figs. 1 and 2). For obtuse angles, the positions of the reflexes are altered as a function of this angle. Fig. 9 shows the dependence of the lattice distances from the angles between the directions a and b (Figs. 1 and 2) of the elementary cell for murein with a twofold screw axis in the peptidoglycan chain and a packing periodicity of $a = 4.5$ Å.

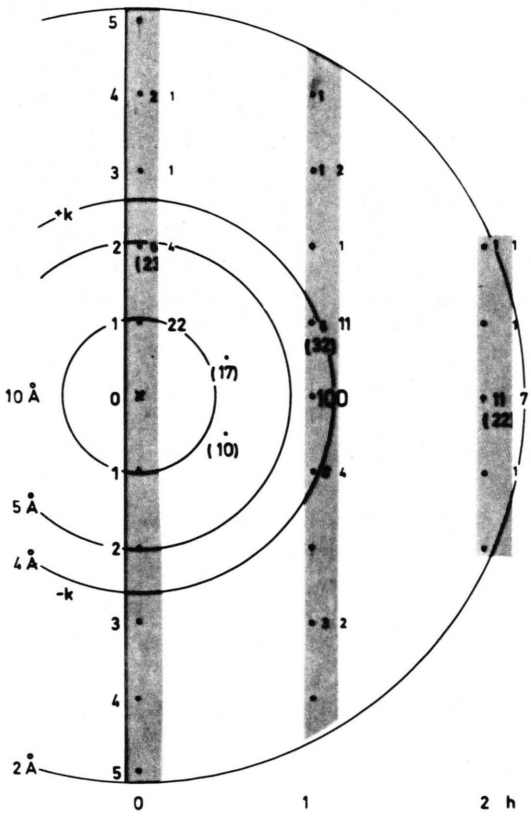

Fig. 8. Calculated two dimensional Fourier-Transform of peptidoglycan chains forming a twofold screw axis (Cellulose- and chitin like conformation). Packing periodicity 4.5 Å. The numbers give intensities in percent of the 1 0 reflex (packing periodicity). First numbers (in dark rows): polysaccharide chains alone; second numbers: polysaccharide chains with peptide chains linked to every second sugar residue.

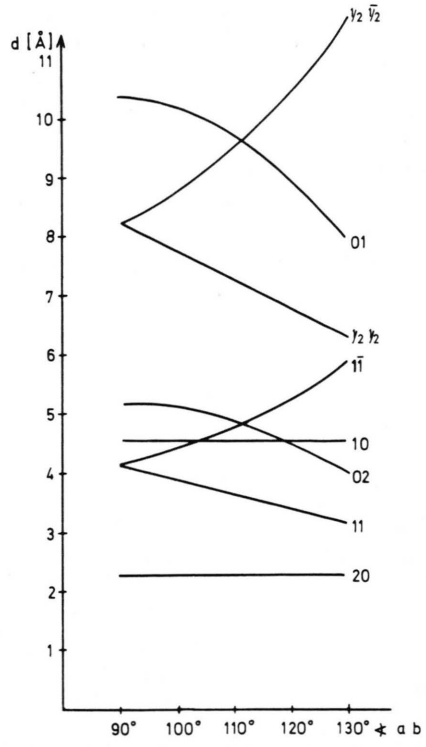

Fig. 9. Dependence of the lattice distances from the angles of the elementary cell for murein with a twofold screw axis in the peptidoglycan chain and a packing periodicity of $a = 4.5$ Å (compare Fig. 1 and 2).

Discussion

Ramachandran type calculations provide two-to threefold screw axes in the sterically allowed region of the polysaccharide chain of murein (Fig. 5). From cross sections of bacterial cells a thickness of about 20 Å was derived for the murein layer of gram negative bacteria [2–4]. Foils prepared from murein sacculi of the gram negative bacterium *Spirillum serpens* provide a Bragg periodicity of 43 Å when viewed edge on [7]. This reflex can be interpreted as thickness of the whole sacculus resulting from head-head or tail-tail superposition of single murein layers with 21.5 Å thickness. Because of this minute thickness of the murein layer, we calculated only two-dimensional fourier transforms of their different possible conformations (Table I). The most intense reflex was always that of the packing periodicity of the peptidoglycan strands (Figs. 7, 8). The higher orders of this reflex occur with reasonable intensities too. Reflexes corresponding to periodicities of about

10 Å can be caused by the repeating periodicities of the peptides along the peptidoglycan chains (Figs. 2, 7, 8) (Table I). If a chitin like structure [24] with a twofold screw axis is assumed for the polysaccharide chain of murein, a narrow packing periodicity of 4.5 Å is possible for these chains. And this distance has been found by several experiments: X-ray diffraction patterns from murein of Gram positive and Gram negative bacteria showed diffuse Debye-Scherrer rings corresponding to Bragg-periodicities of 4.5 and 10 Å [6, 8, 9]. Electron diffraction of thick peptidoglycan of Gram positive bacteria adsorbed onto hydrophilic single crystalline films of graphite oxide cause a diffuse Debye-Scherrer ring in the region of 4.5 Å. This ring could only be obtained, if radiation damage was reduced by using a device which permitted scanning of the specimen for several hours through a focussed electron beam of low current density [11]. Lattice lines 4.5 Å distant from one another could be visualized on the thin murein layer of a Gram negative bacterium by high resolution electron microscopy at 4 K (14). Light optical diffraction of this photograph provided a 4.5 Å reflex together with its higher order at 2.25 Å. The 4.5 Å distance of these lattice lines as well as the 4.5 Å Debye-Scherrer rings of both X-ray and electron diffraction may be interpreted as packing periodicities of the peptidoglycan chains in relatively good agreement with the Fourier transform of Fig. 8, where the 4.5 Å reflex of the packing periodicity is the most intense one.

This narrow chitin like packing is in agreement with the high density values of murein: $\varrho = 1.46$ g/cm³ obtained for murein of the Gram negative Bacterium *Spirillum serpens* and chitin by density gradient measurements [6] and $\varrho = 1.39$ g/cm³ obtained for murein of the Gram positive bacterum *Staphylococcus aureus* by the flotation method [9].

A density value of $\varrho = 1.50$ g/cm³ can be calculated from the following parameters: $a = 4.5$ Å, the packing, periodicity of the peptidoglycan chain; $b = 10.4$ Å, the repeating periodicity of the disaccharide unit assuming a twofold screw axis and a glucosidic angle of 118.5°; $c = 21.5$ Å the thickness of the murein monolayer and a molecular weight of 905 Daltons [6] for the disaccharide-peptide unit of *Spirillum serpens* packed in this elementary cell.

Since the density value calculated for an ideally packed murein is only slightly higher than the experimental value, the agreement is relatively good.

The dense packing of the peptidoglycan chains requires a flat conformation for the polysaccharide and peptide chains. A flat conformation is one, which can be packed with a periodicity of $a = 4.5$ Å

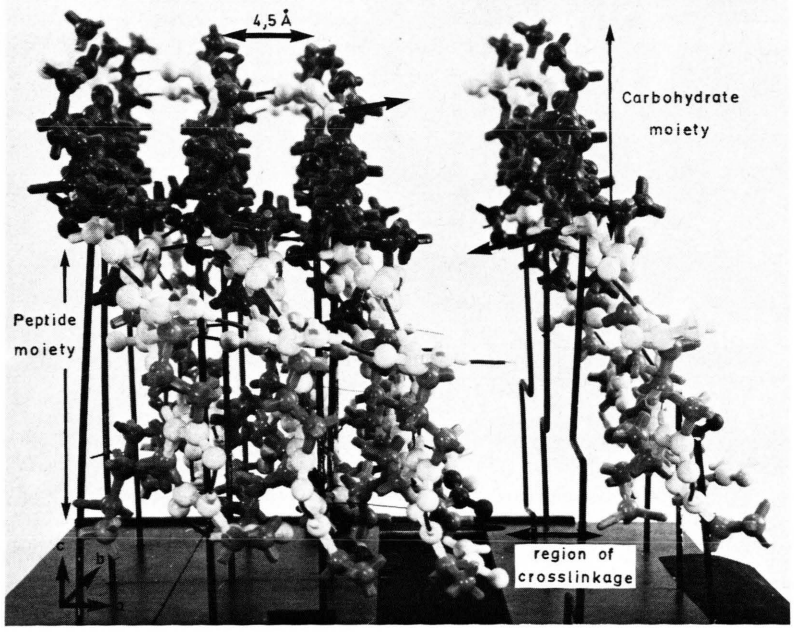

Fig. 10. Three dimensional model of murein. Three peptidoglycan chains are still associated with a periodicity of 4.5 Å. The fourth peptide chain can associate with them by performing further chitin like interchain hydrogen bonds (arrows).

(Figs. 1, 2 and 10) and this is possible, if both polysaccharide and peptide chains perform twofold screw axes.

A twofold screw axis in the polysaccharide chain of murein enables a chitin like structure [24] for the carbohydrate moiety. A twofold screw axis in the hydrogen bonded peptide chain of murein enables two conformations [18]; a pleated sheet [15] and a 2.2_7 helix [6, 13] where the pleated sheet may be excluded by infrared measurements [6, 13].

Calculations of peptide conformations using energy minimization [10] may not provide a naturally occuring conformation, if only one isolated peptide chain is considered and the close contacts between the densely packed peptide chains are neglected.

An argument against the twofold screw axis of the polysaccharide chain of murein and its elementary cell of $4.5 \times 10.4 \times 21.5$ Å^3 may be the Debye-Scherrer ring at about 9.5 Å [6, 8, 9]. An obtuse angle in the elementary cell may, however, cause an X-ray reflex smaller than 10 Å for a real disaccharide length of 10.4 Å (Fig. 9). An further X-ray reflex near 7.5 Å may be caused by the crosslinkage of the peptidoglycan strands. It can be derived from the 1/2 1/2 reflexes at 8.25 Å (Figs. 8, 9).

Two dimensional Fourier calculations of a model of murein based on the chitin like structure of the polysaccharide chains are therefore able to explain the data obtained from density measurements, high resolution electron microscopy, X-ray and electron diffraction. With this model the different modifications of murein [6, 26] its biosynthesis by self-association and its digestion by lysozyme [27] can be explained:

X-ray diffraction on murein foils of Gram positive and Gram negative bacteria taken vertical to the plane of the sacculi showed diffuse Debye Scherrer rings corresponding to distances of about 4.5 and 10 Å [6–10]. Infrared spectra [6, 13] and the results of density gradient measurements on murein of Gram positive [9] and Gram negative [6] bacteria are also similar. Therefore the variations which occur in the mode of crosslinkage and also in the primary structure of the peptide subunits of murein [25] should be compatible with our model. This has really been found by extending model building [26] to examples of all the subgroups of murein described [25]. During the biosynthesis of murein at first an UDP-disaccharide pentapeptide unit is performed. If the peptide chain of this unit is in the 2.2_7, helical configuraton as previously discussed, an optimum of interchain hydrogen bonds can still be performed. Strands resulting from polymerization of these disaccharide pentapeptide units assume a flat conformation and can therefore associate in a similar way as N-acetyl-glucosamin [22] and chitin [24] by performing interchain hydrogen bonds between the N-acetylgroups of adjacent peptidoglycan chains (Fig. 10). The resulting tertiary structure of murein therefore resembles that of chitin, decorated with peptide ribbons protruding into the same direction away from the carbohydrate layer whose repeating periodicities they have to assume (Fig. 2). As a consequence the peptides are brought in a position, that crosslinkage is sterically possible.

The interchain hydrogen bonds and close van der Waals contacts between adjacent peptidoglycan strands are only possible because of the dense packing of the murein structure which is a result of the twofold screw axis both in the polysaccharide and the peptide chains.

Since X-ray diffraction patterns of foils of dried murein showed rather diffuse maxima it seems likely that its structure is somewhat irregular with lattice defects and it is at these sites that lysozyme will act [27]. It has been shown by model building, that in a peptidoglycan lysozyme complex the peptide chains do not exhibit any structural hindrance [27].

Acknowledgement

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