Three-Dimensional Models of the Carbohydrate Moieties of Murein and Pseudomurein

Helmut Formanek

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

Z. Naturforsch. **40c,** 555–561 (1985); received February 28/May 7, 1985

Carbohydrate Chain, Murein, Pseudomurein, Model

Similar density values of $\varrho = 1.39 - 1.46$ g/cm^3 and similar repeating periodicities were determined for murein and pseudomurein. Periodicities of 4.5 and 9.5 Å were measured in the planes of the sacculi of both cell wall polymers. Vertical to these planes periodicities of 42 to 45 Å were obtained.

These data may be interpreted as functions of an oblique-angled elementary cell with an area of about 4.5 Å × 10 Å and a height of 21 to 22.5 Å. This elementary cell includes one disaccharidepeptide subunit. As a consequence of this lattice periodicities the glycan strands of murein and pseudomurein perform twofold screw axes. Therefore the peptide chains, linked at distances of about 10 Å to every second sugar residue, point in identical directions. The parallel array of the polysaccharide chains associated with periodicities of 4.5 Å causes, therefore, a separation of the carbohydrate and peptide moieties of murein and pseudomurein. The head-to-head or tail-to-tail packing of such layers provides periodicities of 42–45 Å.

Because of the twofold screw axis of the glycan strands, the secondary structure of the polysaccharide strands of murein resembles the secondary structure of chitin.

Since all glucosidic oxygen atoms occupy equatorial positions, if the β-1,3 linked N-acetyl-D-glucosamine residues are in the $^4C_1$ conformation and the β-1,3 linked N-acetyl-L-talosaminuronic acid residues in the $^1C_4$ conformation, the polysaccharide chains of pseudomurein may also perform a twofold screw axis.

Introduction

Murein and pseudomurein form the rigid layers of the cell walls of eubacteria [1] and some methanobacteria [2]. These polymers, consisting of polysaccharide chains connected with peptide chains, perform a network structure.

The primary structure of murein is chemically significantly different from pseudomurein. The glycan strand of murein is made up of alternating N-acetyl-D-glucosamine- and N-acetyl-D-muramic acid residues (1) (Fig. 1a, b). The glycan strand of pseudomurein is made up of alternating N-acetyl-D-glucosamine- and N-acetyl-L-talosaminuronic acid residues (2) (Fig. 1c, d). In the case of murein, the peptide chains, consisting of alternating L- and D-amino acid residues, are linked to the carboxylgroups of the D-lactyl residues of muramic acid residues (1) (Fig. 1a, b). In pseudomurein, the peptide chains, consisting only of L-amino acid residues, are linked to the carboxylgroups of talosaminuronic acid residues (2) (Fig. 1c, d).

Reprint requests to H. Formanek.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0700–0555 $ 01.30/0

In spite of these differences in their chemical compositions, the three dimensional structures of both cell wall polymers exhibit similarities which are discussed in the following.

Experimental- and Theoretical Data Available

Considerations about the three dimensional structure of murein and pseudomurein can be based on the data of density measurements, X-ray- and electron diffraction and high resolution electron microscopy.

Density measurements

Similar high density values were obtained for murein [3–5] and pseudomurein [6]. In both cases, values of $\varrho = 1.39$ to 1.46 g/cm^3 were obtained.

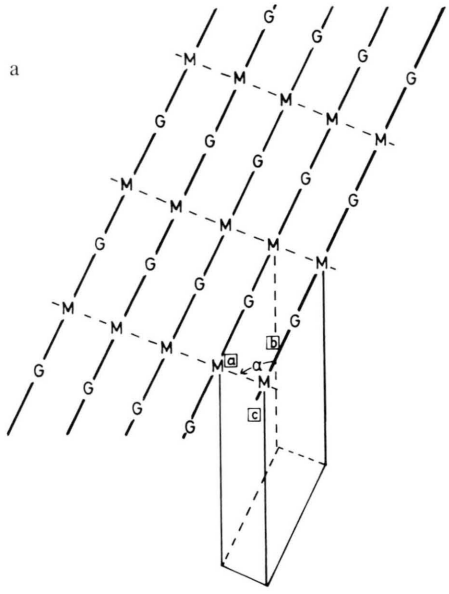
Fig. 1. Scematic drawings of the structures of murein (a, b) and pseudomurein (c, d).
a, c) Network structure with one elementary cell marked.
b, d) Enlarged elementary cell with structure formula.
G = N-Acetyl-D-glucosamine
M = N-Acetyl-D-muramic acid
T = N-Acetyl-L-talosaminuronic acid
a, b, c = Dimensions of the elementary cell;
α = angle between a and b;
Φ, Ψ = torsion angles at the glycosidic oxygen atom.

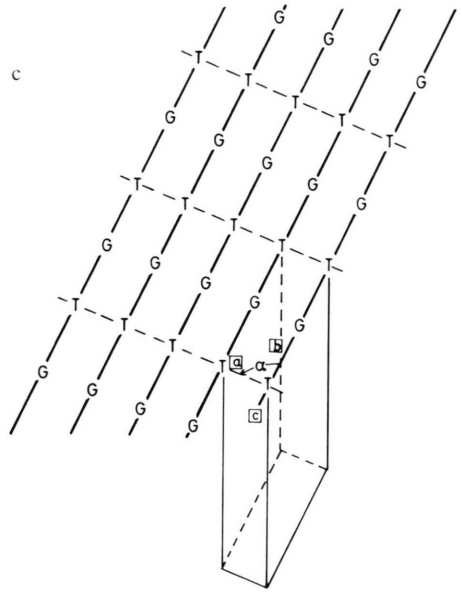
Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

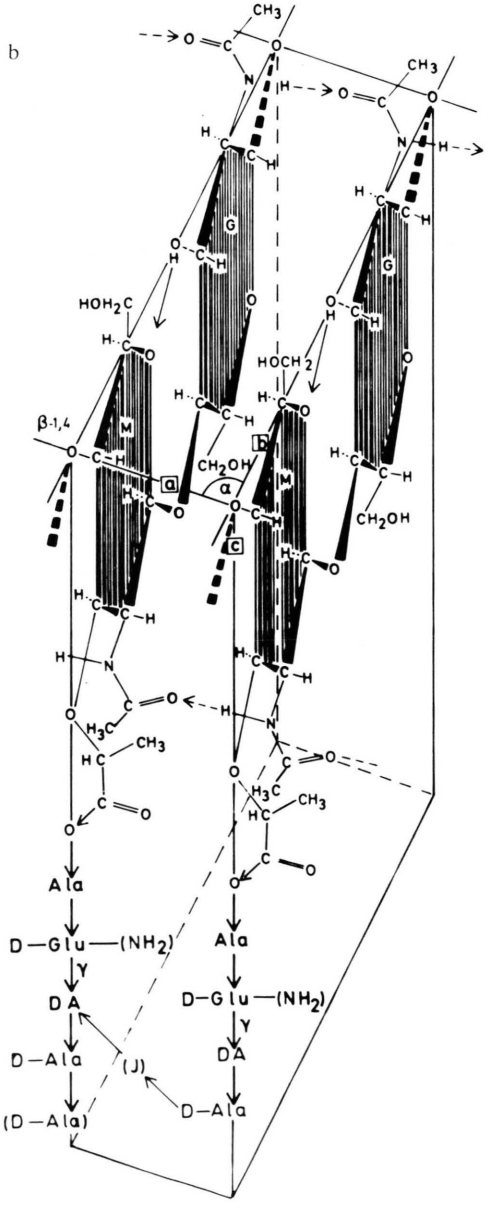
This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

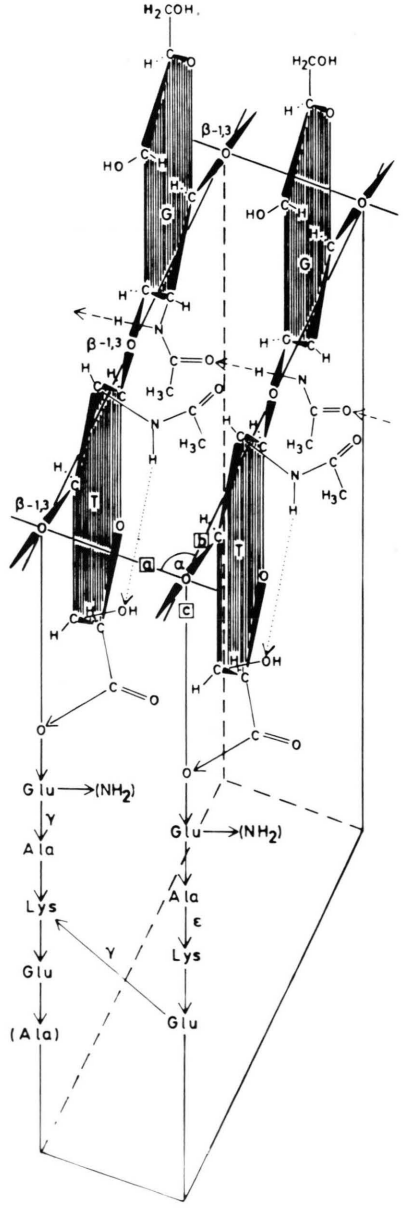
Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.


a
M
G
a
b
c
α


c
T
G
a
b
c
α


b
CH3
HOH2C
HOCH2
CH2OH
β-1,4
α
Ala
D—Glu—(NH2)
γ
DA
D—Ala
(D—Ala)
(J)


d
H2COH
β-1,3
Glu→(NH2)
γ
Ala
Lys
Glu
(Ala)
ε

X-ray diffraction

Foils were prepared from the sacculi of murein of Gram-positive and Gram-negative bacteria [3–5] and the sacculi of the pseudomurein of *Methanobacterium thermoautotrophicum* [6]. The planes of the collapsed sacculi are almost parallel to the planes of the foils.

Diffuse Debye-Scherrer rings corresponding to distances of 4.5 and 9.5 to 10 Å were obtained within the planes of these foils.

Vertical to the planes of the foils of sacculi from the Gram-negative bacterium *Spirillum serpens* a reflexion corresponding to a periodicity of 43 Å has been measured [7]. With the aid of an electron microscope, a thickness of 15 to 30 Å was measured for the murein of Gram-negative bacteria [8–10].

Similar periodicities of 42 to 45 Å vertical to the planes of the foils were found for the murein of the multilayered Gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus* [5] and the pseudomurein of *Methanobacterium thermoautotrophicum* [6].

Electron diffraction and high resolution electron microscopy

The most intense Debye-Scherrer ring obtained by X-ray diffraction, corresponds to a distance of 4.5 Å in the plane of a foild composed of many murein sacculi. This Debye-Scherrer ring was also obtained in the planes of single murein sacculi by electron diffraction [11]. For this purpose the sacculi were adsorbed on hydrophilic, crystalline foils of graphite oxide and a device for the reduction of radiation damage was installed into the electron microscope [12].

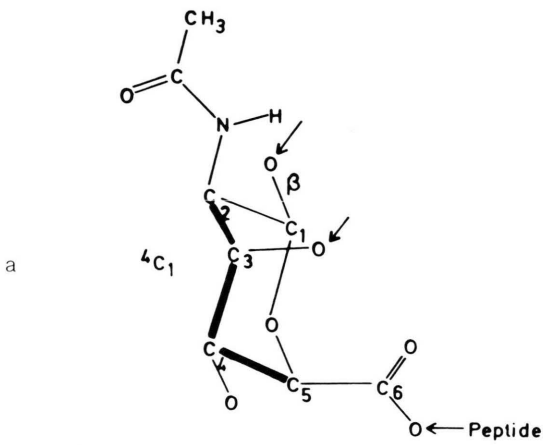
High resolution micrographs taken at 4 K with an electron microscope containing a superconducting lens system [13] provided a fringe structure with a periodicity of about 4.5 Å in the planes of the murein sacculi of *Spirillum serpens* [14]. At 4 K the radiation damage on the object was considerably reduced.

Conformation of the carbohydrate residues

Considerations on the stability of glucopyranose rings [15], energy calculations [15] and X-ray structure analyses on N-acetyl-D-glucosamine [17] and N-acetyl-D-muramic acid [18] proved, that these sugars are always in the $^4C_1$ conformation. For α-D-talose, too, only the $^4C_1$ form was found by X-ray structure analyses [19, 20]. Therefore, it may be concluded that both the mirror symmetric L-talose and N-acetylamino-L-talosaminuromic acid are in the $^1C_4$ form (Fig. 2).

The following comparison between the two theoretically possible conformations $^4C_1$ and $^1C_4$ (Fig. 2) of the N-acetyl-L-talosaminuronic acid residues in pseudomurein may further show why the $^1C_4$ form was taken into consideration for model building.

$^4C_1$ Conformation (Fig. 2a): The N-acetylgroup at C_2 and O_4 are in the sterically unrestricted equatorial



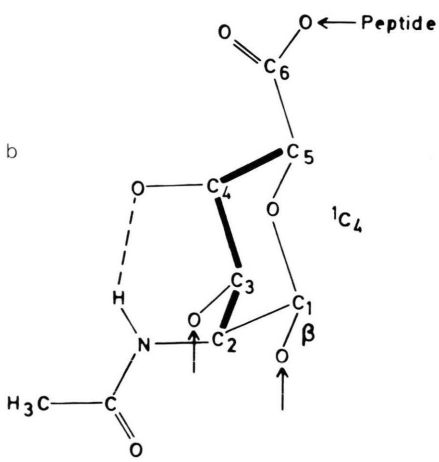


Fig. 2. Chair conformation $^4C_1$ and $^1C_4$ of N-acetyl-L-talosaminuronic acid.
——→ Glycosidically linked oxygen atoms;
— — — → intramolecular hydrogen bond.

positions. The carboxylgroup in position C_6 and O_3 are in axial positions. Since the optimal van der Walls distances for C...C are 3.0 to 3.2 Å and for C...O 2.6 to 2.8 Å, the $^4C_1$ form may be destabilized by three close intramolecular distances:

$C_6....C_1 = 2.8$ Å; $C_6....C_3 = 2.9$ Å; $C_6....O_3 = 2.5$ Å.

A further destabilization effect may be caused by the peptides linked to the bulky carboxyl groups in position C_6 and by the fact that the O_3 atoms are involved in the intrachain glycosidic linkages.

$^1C_4$ Conformation (Fig. 2b): The carboxylgroup in position C_6 to which in pseudomurein, the peptide chain is linked and O_3, which performs the intrachain glycosidic linkage, are in the sterically-unrestricted equatorial position.

The N-acetylgroup at C_2 and O_4 is in axial position. The sterical possibility of an N-acetylgroup or an O_4 in axial position was shown by X-ray structure analyses of N-acetyl-D-mannosamine [21] and N-acetyl-D-galactosamine [22].

The $^4C_1$ conformation of α-D-talose, where both O_2 and O_4 are in axial position, is stabilized by intramolecular hydrogen bonds between O_2 and O_4 [19, 20]. From the X-ray data it can be calculated, that the distance between $O_2....O_4$ is 2.66 Å and the angle between the vectors $O_2....H$ and $O_2....O_4$ is 23 °. Hydrogen bond angles beyond 30 ° are, however, theoretically allowed [23].

Identical hydrogen bonds can be assumed for the mirror symmetric L-talose in the $^1C_4$ conformation and, if the axial O_2 is replaced by an N-acetylgroup, as is the case in N-acetyl-L-talosaminuronic acid, intramolecular hydrogen bonds between the iminogroup and O_4 may be performed with angles between the vectors N-H and $N...O_4$ beyond 30 °.

Proposed structure based on the experimental- and theoretical data

Possible conformation of the glycan strands.

In the β-1,4 linked polyglucosides cellulose, chitin and murein, all glycosidically linked oxygen atoms are in equatorial positions. If, in pseudomurein, the N-acetyl-D-glucosamine residues are in the $^4C_1$ conformation and the N-acetyl-L-talosaminuronic acid residues in the $^1C_4$ conformation, then all glycosidically linked oxygen atoms are also in equatorial position for β-1,3 linkages (Fig. 1d). Equatorial positions for all anomeric O-atoms are, however, obvious, since only axial positions for the anomeric H-atoms were identified by NMR-spectroscopy [24].

In contrast to the 1,4 linkage of murein [1], for pseudomurein the 1,3 linkage [2] is reasonable, because an exchange of N-acetyl-D-glucosamine against N-acetyl-D-galactosamine occuring in several pseudomureins [25], did not introduce any change into the stereochemistry of the glycosidic linkages. The sterical possibilities of the polysaccharide chains of murein were determined, dependent on the rotation angles Φ and Ψ (Fig. 1b), around the bonds to the glycosidic oxygen atoms by using Ramachandran-type calculations [26, 27]. Within the sterically allowed regions of these rotation angles, two to threefold screw axes are possible [26, 27].

Three dimensional models of murein with 2-fold screw axes [3, 28] and models of murein [4, 5] and pseudomurein [6] with two-to-threefold screw axes were therefore proposed.

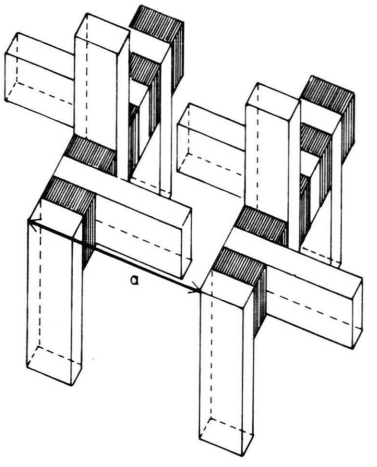
A twofold screw axis provides the highest value for the disaccharide peptide periodicity in the direction of the polysaccharide strand (b in Fig. 1) [27]. With a screw axis higher then twofold this value can be reduced in murein from 10.3 Å to 9.4 Å if a right angled elementary cell (α = 90 ° in Fig. 1) is considered for the murein network [27]. On the other hand an obtuse angled elementary cell (α > 90 ° in Fig. 1) can also cause a 9.5 Å reflex (b × sin α in Fig. 1) for the 10.3 Å periodicity (b in Fig. 1) caused by the twofold screw axis. Similar considerations are valid for pseudomurein.

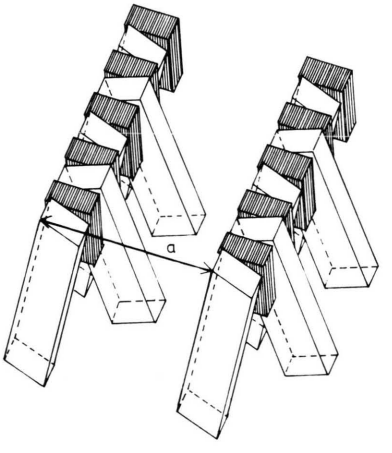
In the case of non-twofold screw axis, the peptide chains are radially distributed around the polysaccharide strands [5, 6] (Fig. 3a). A 2.66-fold screw axis, for example, causes right angles between the peptide chains linked to every second sugar residue of the polysaccharide strands.

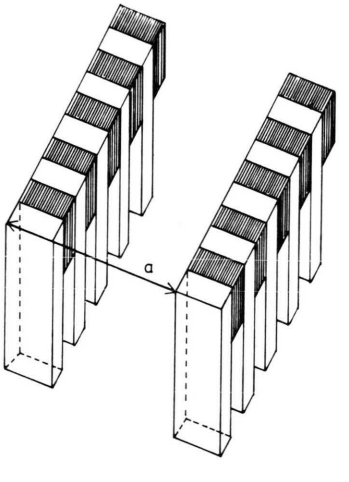
Only in the case of a twofold screw axis or a screw axis oscillating around the twofold symmetry, do the peptide chains point in identical or almost identical direction (Fig. 3b, c) [27] causing thereby the separation of the carbohydrate and peptide moieties.

Elementary cell

Two dimensional Fourier-transforms were calculated for murein networks with different packing periodicities (a in Fig. 1) with the results, that the packing periodicity provided always the strongest reflexion [27]. These calculations are also valid for







pseudomurein networks. Therefore, the strongest Debye-Scherrer ring corresponding to a distance of 4.5 Å in the planes (a, b in Fig. 1) of murein and pseudomurein sacculi may be interpreted as a function ($a \times \sin \alpha$) of the packing periodicity of their glycan strands, while the Debye-Scherrer ring at about 9.5 Å may be explained as a function ($b \times \sin \alpha$) of the repeating periodicity of the disaccharide peptide units along the polysaccharide strands.

The periodicity of 42 to 45 Å measured vertical to the planes of the sacculi of murein and pseudomurein may be interpreted in two different ways.

1. For screw axes of the polysaccharide strands higher than twofold (Fig. 3a) where the peptide chains are radially oriented around the glycan strands, three to four individual layers may be needed to form the periodicity of 42 to 45 Å [5, 6].

2. For a twofold screw axis (Fig. 3c) or a screw oscillating around the twofold symmetry (Fig. 3b), the periodicity of 42 to 45 Å may be caused by two head-to-head or tail-to-tail superimposed murein layers of 21 to 22.5 Å thickness [3, 27].

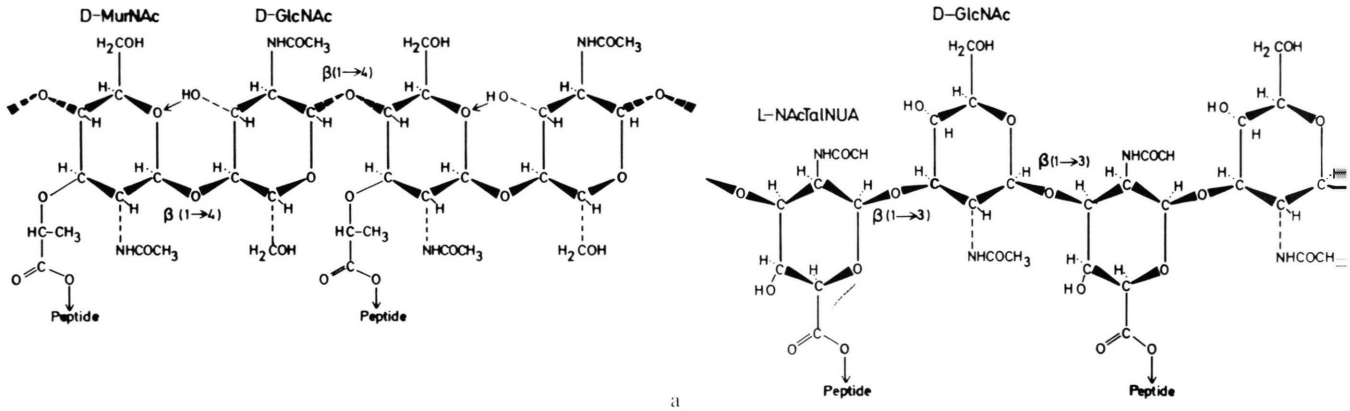
In the case of a twofold screw axis, the two distances measured in the planes of the sacculi of murein and pseudomurein and one distance measured vertical to this planes may be interpreted as function of the dimensions, a, b, c (Fig. 1) of the elementary cells of murein and pseudomurein, where each elementary cell contains one disaccharide peptide unit.

Since the density values of about 1.5 g/cm^3 calculated with these data for an ideally single crystalline structure are only slightly higher than the measured values of 1.39 to 1.46 g/cm^3 for the partially disordered paracrystalline structure of murein and pseudomurein, the agreement is relatively good.

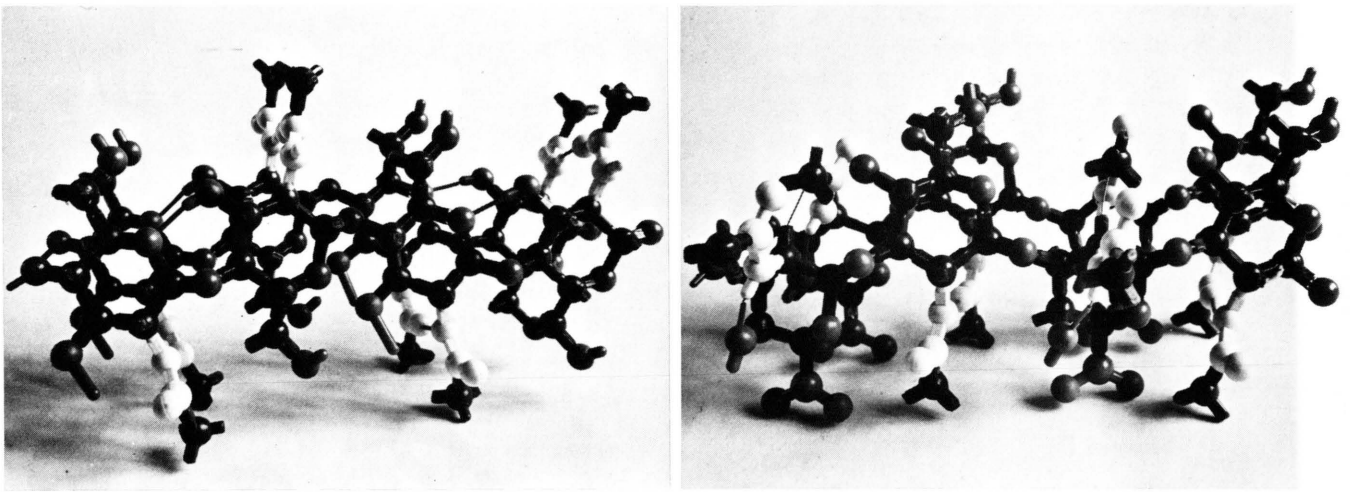
Model building

By model building packing periodicities of about 4.5 Å (a in Fig. 1) are possible for glycan strands of murein and pseudomurein with twofold screw axes (Fig. 4). This narrow packing was also found in chitin

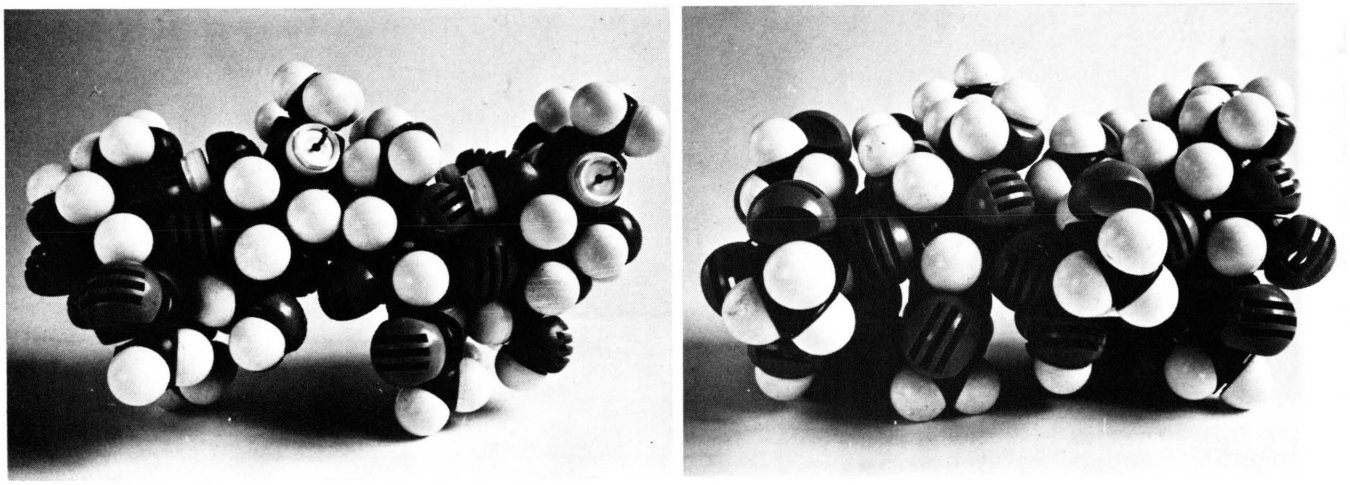
Fig. 3. Sceme of screw axes theoretically possible in the polysaccharide strands of murein and pseudomurein. Short beams symbolize N-acetyl-D-glucosamine residues. Long beams symbolize N-acetyl-D-muramic acid- or N-acetyl-L-talosaminuronic acid residues with linked peptide chains.
a) 2.66-fold screw axis;
b) screw axis oscillating around a twofold symmetry;
c) 2-fold screw axis.



a



b



c

Fig. 4. Tetrasaccharide from the polysaccharide chain of murein and pseudomurein.
a) Structure formula; b) transparent model; c) space filling model. b, c) Two tetrasaccharide chains, packed behind one another with a periodicity corresponding to a distance of 4.5 Å.

by X-ray structure analysis [29]. It permits interchain hydrogen bonds between the N-acetylgroups of the N-acetyl-D-glucosamine residues of both murein and pseudomurein and, in the case of murein, also between the N-acetylgroup of N-acetyl-D-muramic acid residues (Figs. 1 and 4). In pseudomurein, the axially oriented N-acetylgroups of the N-acetyl-L-talosaminuronic acid residues cannot perform interchain hydrogen bonds since their NH-groups are still involved in intramolecular hydrogen bonds with the axially-oriented O_4-atoms (Fig. 2). In addition, the axially-oriented N-acetylgroups are "overlapping" with the N-acetyl-L-talosaminuronic acid residues of the adjacent polysaccharide strands (Fig. 4). This "overlapping" is sterically possible because of the "zigzag structure" of the polysaccharide chain of pseudomurein (Fig. 4), where the N-acetyl-L-talosaminuromic acid residues sit "deeper" than the N-acetyl-D-glucosamine residues.

Conclusions

The model discussed for murein and pseudomurein is a simple extension of the two dimensional representation of a network to a thin three dimensional layer (Fig. 1). Two distances measured in the plane of this layer and one distance vertical to it may be explained as the three dimensions of an elementary cell. This includes one disaccharide peptide unit, the smallest repeating unit of the layer.

Acknowledgements

I thank Prof. Dr. E. Th. Rietschel, Forschungsinstitut Borstel and Prof. Dr. F. Fiedler, Institut für Genetik und Mikrobiologie der Universität München for critically reading the manuscript. This work was supported by grant number Fo 106/3 of the Deutsche Forschungsgemeinschaft.

[1] W. Weidel and H. Pelzer, Adv. Enzymol. **26,** 192 (1964).
[2] O. Kandler and H. König, Arch. Microbiol. **118,** 141 (1978). O. Kandler, Naturwissenschaften **66,** 95 (1979).
[3] H. Formanek, S. Formanek, and H. Wawra, Eur. J. Biochem. **46,** 279 (1974).
[4] R. E. Burge, A. G. Fowler, and D. A. Reavely, J. Mol. Biol. **117,** 927 (1977).
[5] H. Labischinski, G. Barnickel, H. Bradaczec, and P. Giesbrecht, Eur. J. Biochem. **95,** 147 (1979).
[6] H. Labischinski, G. Barnickel, B. Leps, H. Bradaczek, and P. Giesbrecht, Arch. Microbiol. **127,** 195 (1980).
[7] H. Wawara, Z. Naturforsch. **31c,** 635 (1976).
[8] S. de Petris, J. Ultrastruct. Res. **19,** 45 (1967).
[9] H. Frank and D. Dekegel, Folia Microbiol. **12,** 227 (1967).
[10] H. P. Hofschneider and H. H. Martin, J. Gen. Microbiol. **51,** 23 (1968).
[11] H. Formanek and G. Rauscher, Ultramicroscopy **4,** 337 (1979).
[12] G. Rauscher and H. Formanek, USA Patent 4376891 (1983).
[13] I. Dietrich, F. Fox, E. Knapek, G. Lefrac, K. Nachtrieb, R. Weyl, and H. Zerbst, Ultramicroscopy **2,** 241 (1977).
[14] I. Dietrich, H. Formanek, F. Fox, E. Knapek, and R. Weyl, Nature **277,** 380 (1979).
[15] R. E. Reeves, J. Am. Chem. Soc. **72,** 1499 (1950).
[16] V. S. R. Rao, P. R. Sundararjan, C. Ramakrishnan, and G. Ramachandran in Conformation of Biopolymers, **Vol. 2** Ed. p. 721, Acad. Press, New York 1967.
[17] L. N. Johnson, Acta Cryst. **21,** 885 (1966).
[18] J. R. Knox and N. S. Murthey, Acta Cryst. **B 30,** 365 (1974).
[19] L. K. Hansen and A. Hordvik, Acta Chem. Scand. **A 31,** 187 (1977).
[20] P. J. Ohanessian, D. Avenel, J. A. Kanters, and D. Smits, Acta Cryst. **B 33,** 1063 (1977).
[21] P. A. Neuman, H. Gillier-Pandraud, and F. Longchambon, Acta Cryst. **B 31,** 2628 (1975).
[22] R. D. Gilardi and J. L. Flippen, Acta Cryst. **B 30,** 2931 (1974).
[23] L. Pauling, R. B. Corey, and H. R. Branson, Proc. Nat. Acad. Sci. USA **37,** 205 (1951).
[24] H. König, O. Kandler, M. Jensen, and E. Th. Rietschel, Hoppe-Seyler, Z. Physiol. Chem. **364,** 627 (1983).
[25] H. König, R. Kralik, and O. Kandler, Zbl. Bakt. Hyg., I. Abt., Orig. **C 3,** 179 (1982).
[26] G. N. Ramachandran, G. Ramakrishnan, and V. Sasisekharan, in Aspects of Protein Structure (G. N. Ramachandran, Ed.) p. 121, Acad. Press, New York 1963.
[27] H. Formanek, Z. Naturforsch. **37c,** 226 (1982).
[28] M. V. Kelemen and H. J. Rogers, Proc. Nat. Acad. Sci. USA **68,** 922 (1971).
[29] D. Carlström, J. Biophys. Biochem. Cytol. **3,** 669 (1957).