Three-Dimensional Molecular Models of Bacterial Cell Wall Mucopeptides (Peptidoglycans)

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ABSTRACT Molecular models have been built of mucopeptides (peptidoglycans) from various species of bacteria. If pairs of glycan chains are hydrogen-bonded as are those of chitin, the carboxyl groups of muramic acid are so oriented that a regular three-dimensional structure can be built. Helical conformation of the peptides is not likely, but pseudo-β conformation gives the possibility of multiple interpeptide hydrogen bonding. Calculations of the expected dimensions of the cell wall from the model for mucopeptide give results of the right order for bacilli. The effect of the configuration of the amino acids on the conformation of the peptide has been examined.

The mucopeptides (peptidoglycans) are insoluble polymers present in almost all bacterial cell walls. They maintain the shape of bacteria and protect the cell against the strong osmotic forces which in their absence burst the membranes underlying the walls. They are also necessarily involved in an important way in the growth and division of bacteria (1). The chemical structure of several mucopeptides has been elucidated during the last decade (2, 3); they consist of polysaccharide chains of various lengths, linked together by peptides consisting of a limited range of amino acids. The polysaccharides always consist of N-acetylmuramic acid and N-acetylgucosamine linked together by 1 → 4 β-glycoside linkages. The peptides differ from one species of microorganism to another but usually do not involve more than four amino acids, some in the less-usual D-configuration. D-Alanine and D-glutamic acid seem always to be present. A number of two-dimensional ways of illustrating these rather complicated polymers have been attempted, mostly reflecting convenience rather than attention to molecular parameters (2-7). To try to build precise, unambiguous models of these three-dimensional insoluble polymers based on calculation of the minimum energies of bond rotation and nonbonded interactions, as has been done for simple soluble peptides (8-11), would be extremely difficult and perhaps not very logical. We therefore decided to examine the range of likely conformations that might be adopted, particularly by the mucopeptides from Gram-positive species of bacteria, where the thick cell walls necessarily imply several layers of the polymers. Examination of the arrangement in the walls of these polymers by orthodox physical techniques such as IR spectroscopy, x-ray diffraction, and optical rotatory dispersion is difficult, and it seemed therefore justifiable to build molecular models at this stage. The models suggest new tests of properties of the mucopeptides for consistency with the conformations in the models.

MATERIALS AND METHODS

The atomic models were designed by Mr. Frank Dore of this Institute and were inexpensive—a necessity with the large models required for the present work. The scale of the models was 1 Å = 1.75 cm. The bond lengths adopted were C-C, 1.53 Å; C=O, 1.24 Å; and C—N, 1.32 Å. The hydrogen bonds were all taken as 2.83 Å. The models were supported on steel pillars fixed into large, perforated baseboards. The oligosaccharide chains were arranged horizontally (parallel to the baseboards) and the peptides were run between them. Bonding is described as being horizontal or vertical, according to whether it is approximately parallel to the baseboard or at right angles to it (Fig. 1).

**Fig. 1.** Diagrammatic representation of the way in which the models were constructed and the significance of the terms horizontal and vertical used in the text.
keep the mucopeptide structure expanded by electrostatic interactions.

In view of these high concentrations, the mucopeptide units are likely to be packed closely together. The polysaccharide chains are formally similar to those in chitin, which is a homopoly saccharide of 1 → 4 β-linked N-acetylglucosamine. We therefore built models with the polysaccharide chains arranged head to tail and with the N-acetylglucosamine residues hydrogen-bonded together, as are those of chitin (15). Two consequences of such an interchain arrangement were apparent: (i) all the carboxyl groups of N-acetylmuramic acid protrude from the same side of a stack of polysaccharide chains, (ii) the six hydroxyl groups of this sugar derivative are exposed on the opposite side of the stack. It is to the carboxyl groups that the peptides are attached, and to the six hydroxyl positions that wall polymers other than mucopeptide are likely to be joined. When polysaccharide chains were arranged in this way, the distance between —NH·CO·CH₃ in neighboring polysaccharide chains was 2.6 Å. The length of an octasaccharide in a single chain was 41.1 Å and the carboxyl groups of the muramyl residues were 10.3 Å apart. The cross-section of a single chain was about 7 Å. The last three measurements would not be modified by altering the interchain arrangement and hydrogen bonding, but the relative angles of the carboxyl groups in different chains would be altered. Since the peptides are attached to these groups, alteration of the angles of protrusion would lead to quite different overall arrangements of the mucopeptide. Oligosaccharide chains of up to 10 units were relatively stiff, and if they were hydrogen-bonded as in chitin, two or three chains together were almost inflexible.

Conformation of the peptide chains

The peptide chains in all mucopeptides differ from those of proteins in four respects: (a) some of the amino acids are in the d configuration; (b) d-glutamic acid, which always occurs, and d-aspartic acid, which sometimes occurs, have their γ- and β-carboxyl groups, respectively, involved in the peptide linkage, and not their α-carboxyl groups; (c) some of the diaminos, one of which always occurs (for example, L-lysine and meso-diaminopimelic acid), are linked into the peptides by both amino groups; (d) the peptides linking two oligosaccharide chains are made of two parts joined head to tail with respect to their amino and carboxyl terminal groups. These points are all illustrated in Fig. 2. The effect of these
各种差异表明，肽的构象变化。氨基酸残基（a）的变化，在各种肽聚糖模型中，变化极小，且无任何非共价相互作用，因此不能画出任何明显的结论。由（b）和（c）可知，二价和二胺酸残基存在于肽链中，结果表明，未充分延伸的α-螺旋形成氢键。在可比较的肽聚糖中，α螺旋的正常蛋白质中未见氢键。氢键较短，长度为10-12个氢键。α-螺旋结构由不溶性肽聚糖链（a）营养无螺旋肽聚糖非常不规则。

我们把考虑两种其他可能的构象，即随机环或半-β排列。随机环保持稳定性，但若非糖残基不紧密堆叠。如在图4中所示，随后，若非糖残基堆叠，甚至在二环中两层厚（参照图5）时，聚糖仍为不定规。这样氢键被占据，而氢键的相互作用为横行和纵向两层厚（参照图5），则聚糖必须至少延伸以占据空间的厚度。这是因为相对密度较低（12）。因此我们对β-像构象给以相当的注意。真β构象若无可能，因为非糖残基的二价与二胺酸链在肽聚糖链中。当模型被制作时，非糖残基链排列如在β-和肽聚糖中的肽聚糖中的肽聚糖，它表明，肽链高比例的肽聚糖不能在连续的链中以适合位置排列。

图3. 模型的直链肽聚糖。肽链在相反方向，与未被取代的2,6-二胺基顺酸侧链在相反侧的模型。图中的虚线代表潜在的氢键。图中所示的肽聚糖残基实际上为N-乙酰化。当氢键，形成α-螺旋不能发生。例如，在金黄色葡萄球菌肽聚糖中，十七个肽链中涉及，包括这些碳基中的二价和二胺酸链的乳酸侧链和L-丙氨酸残基，但只有三种氢键在肽聚糖中。在可比较的肽聚糖中，α-螺旋的正常蛋白质中未见氢键。氢键较短，长度为10-12个氢键。α-螺旋结构由不溶性肽聚糖链（a）营养无螺旋肽聚糖非常不规则。

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The head-to-tail arrangement of the cross-linking peptides [(d) above] was another reason for the small number of hydrogen bonds formed in helical arrangements, and in some instances led to differences in the degree of hydrogen bonding between neighboring peptides according to the orientation of the individual peptides. For example, a center of asymmetry exists in the heptapeptide of the bacillary mucopeptide, caused by the inclusion of both amino groups of one residue of meso-diaminopimelic acid but only one of the second residue. Most hydrogen bonding is likely when the free meso-diaminopimelic acid side chain is toward opposite ends of neighboring peptides (Fig. 3).

Although ionic forces did not seem to be likely to play much part in most mucopeptides, they were a possibility in the mucopeptide from Micrococcus luteus. In this organism the bridge consists of 0-4 head-to-tail arrangements of the tetrapptide-L-Ala-d-Glu(Gly)-l-Lys(16). When the models were made, we found that if the peptides were in pseudo-β conformation the free carboxyl group of the glycerol residue that is substituted on to the α-COOH groups of the d-glutamyl residues came extremely close to the free ε-NH₂ groups of L-lysyl residues in the next peptide chain. This may account for the greater alterations in the dextran-impermeable volume (17) of walls from M. luteus (compared with those from staphylococcus) when the surrounding pH or ionic strength is changed, despite the large negative charge borne by the walls of staphylococcus caused by the teichoic acids attached to the mucopeptide.

As a first hypothesis, one may suppose that the space occupied by the mucopeptide in the wall is controlled principally by two interrelated factors: the packing of the oligosaccharide chains and the conformation of the peptides attached to them. The conformation of the rather short polysaccharide chains themselves is not likely to be a dominant factor. If we assume some degree of close packing of the oligosaccharide chains (as in chitin), we can examine how much space would be occupied by the mucopeptide with the peptides extended in β conformation. Two simple arrangements of the oligosaccharide can be imagined for rod-shaped bacteria. One is that oligosaccharide chains are packed radially as discs two chains thick (see Fig. 4) and the other is that the chains run either longitudinally or circumferentially along the wall in layers two chains thick (see Fig. 5). From the point of view of the calculations these two possibilities can be regarded as one, since the length and circumference of the bacilli used for the calculations are not very different. From a knowledge of the amount of mucopeptide per
individual cell and the molecular parameters taken from the models, it is possible to calculate the length of the cell from the first type of arrangement and the thickness of the wall necessary to accommodate the mucopeptide from the second. The results obtained for the calculated dimensions are of the right order (see Table 2). The agreement is not impressive but, although it is not possible to distinguish between the two sorts of arrangement of mucopeptide, it is possible to say that a reasonable amount of the peptide could well be in an extended conformation. It must be remembered that these calculations include the number of bacteria per unit mass, and counting of bacilli by orthodox methods is notoriously inaccurate because of the failure of the micro-organisms to separate. It may be noted that one of the calculations gives a very low result for the length of the cell, while two give somewhat higher results. The high results may provide some argument against all the peptide being in an extended conformation. Measurement of the volume occupied by the mucopeptide alone in B. subtilis 168 shows that this would be about 40% of the measured volume of the wall when packed as tightly as possible. Thus, considerable dispersion of the mucopeptide units in the wall is unlikely, and it also seems somewhat unlikely that the great reduction in thickness of walls seen (14) when the polymers other than mucopeptides are removed indicates a true disposition of the mucopeptide in the untreated wall. The possible collapse of the mucopeptide when deprived of its negatively charged polymers may be part of the explanation for the ubiquitous presence of strongly negatively charged polymers such as the teichoic and teichuronic acids in bacterial cell walls.

Further evidence is required, but the models suggest relevant experiments. Measurements of the dimensional changes of mucopeptide particles freed of other polymers such as the teichoic and teichuronic acids, according to the ambient pH, ionic strength, presence of polyvalent cations (18), and presence or absence of agents (such as urea or guanidine hydrochloride) that weaken hydrogen bonds, might provide some evidence as to the proportion of hydrogen and ionic bonding. This differs greatly in the different models. Finally, further evidence of the permeability of mucopeptide to small neutral molecules (19), particularly if studied under different conditions of pH and ionic strength, and in the presence and absence of reagents that weaken hydrogen bonds, might give evidence of the degree of packing of the mucopeptide units in the walls.

The amount of mucopeptide per unit weight of microorganisms was estimated for Bacillus subtilis 168 ind⁻¹ by direct estimation of the amount of diaminopimelic acid in acid-hydrolyzed trichloroacetic acid-extracted bacteria; for B. licheniformis 6346 it was estimated from the analysis of isolated walls and the yield of walls obtained. Cell counts were made in a Coulter counter with a 70-µm orifice. The dimensions of the bacteria were measured on living cells using a Dyson split-image eyepiece and the wall thickness was measured from a large number of electron microscope pictures of sections.

<table>
<thead>
<tr>
<th>Cell length (µm)</th>
<th>Cell radius (µm)</th>
<th>Measured thickness of wall (Å)</th>
<th>Mucopeptide/100 mg cell (µmol)</th>
<th>Mucopeptide per cell (µmol)</th>
<th>Total length of polysaccharide per cell (µm)</th>
<th>No. of chains packed Along cell</th>
<th>Radially</th>
<th>Calculated Wall thickness (Å)</th>
<th>Calculated Cell length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis 168 ind⁻¹ (grown on minimal medium)</td>
<td>2.67</td>
<td>0.341</td>
<td>195–300</td>
<td>11</td>
<td>1.85 × 10⁻¹⁰</td>
<td>4.3 × 10⁴</td>
<td>3.9–5.6 × 10⁶</td>
<td>305</td>
<td>2.82–3.91</td>
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<tr>
<td>Bacillus licheniformis 6346</td>
<td>2.9</td>
<td>0.341</td>
<td>380–500</td>
<td>7.2</td>
<td>1.05 × 10⁻¹⁰</td>
<td>6.35 × 10⁴</td>
<td>2.45 × 10⁴</td>
<td>1.4–1.7 × 10⁶</td>
<td>210</td>
</tr>
<tr>
<td>Bacillus subtilis 168 ind⁻¹ (grown on casein hydrolysate medium)</td>
<td>3.38</td>
<td>0.395</td>
<td>300–380</td>
<td>7.4</td>
<td>3.7 × 10⁻¹⁰</td>
<td>2.30 × 10⁴</td>
<td>3.31 × 10⁴</td>
<td>6.50 × 10⁶</td>
<td>350</td>
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