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*-acetylglucosamine linked together by 1 $\rightarrow$ 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).

Packing of the oligosaccharide chains in the wall

The concentration of material in bacterial walls is relatively high. In *Bacillus megaterium*, for example, the dextran-impermeable volume of the wall is 41% of the cell volume, while the dry weight forms a proportion of 28% (12). In *Bacillus subtilis* ind⁻ 168, the wall forms 39% of the volume of the cell as calculated from electron microscope pictures of sectioned microorganisms, and the wall forms 20% of the dry weight according to estimations of the diaminopimelic acid content of acid hydrolysates of whole, trichloroacetic acid-extracted bacteria. In these two examples the concentration of solids in the wall amounts to 69 and 51 g/100 ml. The walls consist of only about 40% of their weight as mucopeptide (13), but the thickness of the wall remaining after the removal of the other polymers present (which can be done without measurable chemical change in the mucopeptide) is also smaller by about 50% (14). It is not clear whether the observed reduction in wall thickness is a true indication of the distribution of mucopeptide and other polymers in the walls, or whether the mucopeptide structure collapses during preparation for electron microscopy as a result of the removal of the other polymers, which are strongly negatively charged and may help to

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 homopolysaccharide 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 ap-

FIG. 2. Diagram from the model of the mucopeptide from *Staphylococcus aureus*. The glycan chains are running through the plane of the paper; the A, B, and C chains were vertically above one another in the model. The peptides were in β configuration, with the pentaglycyl bridges running in the vertical plane. The dotted lines represent potential hydrogen bonds formed in the model.

parent: (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 $-\text{NH} \cdot \text{CO} \cdot \text{CH}_3$ 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 diamino acids, 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

various differences on the conformation of the peptides has been examined. The effect of changing the configuration of the amino acids [(a) above] in the various mucopeptide models was surprisingly slight and, without being able to allow for non-bonded interactions, it was not possible to draw any obvious conclusions. The consequences of (b) and (c) are that the methylene groups of the dibasic and diamino acids are included in the peptide chain and the results of this are clearest when a helical conformation is attempted. Even though a number of the peptides are long enough to make reasonable

FIG. 3. Diagram from the model of the bacillary mucopeptide. The peptides run in opposite directions, with the unsubstituted 2,6-diaminopimelic acid side-chains on opposite sides of the model. The broken lines represent potential hydrogen bonds. The muramic acid residues shown are actually *N*-acetylated in the mucopeptide.

helices, sufficient hydrogen-bond formation to stabilize the α -helix cannot occur. For example, in staphylococcal mucopeptide, seventeen peptide bonds are involved, including those between the carbonyl groups of the lactyl side chains of the *N*-acetylmuramic acid residues and the *L*-alaninyl residues, but only three hydrogen bonds were likely within the peptide. In the comparable polypeptide α -helix of normal protein there would be 10–12 hydrogen bonds. The helix-like structure formed by the staphylococcal peptide was very irregular indeed.

We were thus led to consider two other possible conformations, namely random loops and a pseudo- β arrangement. Random loops remain a possibility, but only if the oligosaccharides are not tightly packed. As will be seen later, if the oligosaccharides are packed, even in discs two chains thick (see Fig. 4) or longitudinal layers two chains thick (see Fig. 5), then the peptides must be at least partly extended to occupy the space of the wall and to account for their relatively low density (12). For this reason we gave considerable attention to the β -like conformation. True β conformation was again not possible because of the inclusion of the methylene groups from dibasic and diamino acids in the peptide chains. When the models were made with the oligosaccharide chains arranged as in chitin and the peptides in the pseudo- β conformation, it was apparent that a high proportion of the peptide groups in contiguous chains would be so positioned as to be likely

to form hydrogen bonds (see Table 1). Such multiple hydrogen bonding could contribute significantly to the physical properties of the mucopeptides, as it does to other insoluble polymers such as chitin, cellulose, and collagen.

In some mucopeptides with cross bridges, such as that from staphylococci (Fig. 2), it was possible for the bridges to run from one set of neighboring peptides to another, either in a horizontal or vertical direction in the model. Notable differences in the degree of hydrogen bonding were apparent according to this orientation of the cross bridges (see Table 1).

TABLE 1. Calculated percentage of hydrogen bonding in different mucopeptide models

Source of mucopeptide	Length of peptide (Å)	Carbonyl groups per cross-linked chain		Imino groups per cross-linked chain		% Total >CO + >NH H-bonded
		Total	H-bonded	Total	H-bonded	
<i>Bacillus</i>						
Helical		13	2	9	4*	27
Pseudo- β	46.8	13	5	9	5	46
<i>Staphylococcus aureus</i>						
Helical		17	3	17	5*	24
Pseudo- β	61.6	17	7	17	7	41
		17	13	17	13	77
<i>Corynebacterium poinsettiae</i>						
Pseudo- β	41.1	13	6	10	6	52
		13	8	10	8	70
<i>Micrococcus luteus</i>	87.0	26	11	24	12	66

The carbonyl and imino groups included in the calculation were those found in two peptides that could be cross-linked by the carboxyl of a D-alanine or a bridge peptide. The carbonyl groups of muramic acid were included. Lengths of peptides are calculated from one carbonyl group of the lactyl side-chain of *N*-acetylmuramic acid to another.

* Two >NH groups are H-bonded to each >CO group in *N*-acetylglucosamine.

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 tetrapeptide-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 glycyl 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

FIG. 4. One hypothetical arrangement of mucopeptide within the wall of a rod-shaped Gram-positive microorganism. The radial lines represent glycan chains; the longitudinal lines, peptide.

FIG. 5. Another hypothetical arrangement of mucopeptide within the wall of a rod-shaped Gram-positive microorganism. The longitudinal lines represent glycan chains; the radial lines, peptide.

TABLE 2. Fit of mucopeptide units into the dimensions of the walls of two *Bacillus* species with peptides extended in pseudo- β conformation

	Cell length (μm)	Cell radius (μm)	Measured thickness of wall ($\text{\AA}$)	Mucopeptide/100 mg cell (μmol)	Mucopeptide per cell (μmol)	Total length of polysaccharide per cell (μm)	No. of chains packed		Calculated	
							Along cell	Radially	Wall thickness ($\text{\AA}$)	Cell length (μm)
<i>Bacillus subtilis</i> 168 ind ⁻ (grown on minimal medium)	2.67	0.341	195–300	11	1.85×10^{-10}	1.15×10^5	4.3×10^4	$3.9\text{--}5.6 \times 10^5$	305	2.82–3.91
<i>Bacillus licheniformis</i> 6346	2.9	0.341	380–500	7.2	1.05×10^{-10}	6.35×10^4	2.45×10^4	$1.4\text{--}1.7 \times 10^5$	210	1.05–1.42
<i>Bacillus subtilis</i> 168 ind ⁻ (grown on casein hydrolysate medium)	3.38	0.395	300–380	7.4	3.7×10^{-10}	2.30×10^5	3.31×10^4	6.50×10^5	350	4.20

The amount of mucopeptide per unit weight of microorganisms was estimated for *Bacillus subtilis* 168 ind⁻ 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.

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.

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