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Structure, Biochemistry and Mechanism of Action of Glycopeptide Antibiotics

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Glycopeptide antibiotics, including vancomycin and teicoplanin, are large, rigid molecules that inhibit a late stage in bacterial cell wall peptidoglycan synthesis. The three-dimensional structure contains a cleft into which peptides of highly specific configuration (L-*aa*-D-*aa*-D-*aa*) can fit: such sequences are found only in bacterial cell walls, hence glycopeptides are selectively toxic. Glycopeptides interact with peptides of this conformation by hydrogen bonding, forming stable complexes. As a result of binding to L-*aa*-D-Ala-D-Ala groups in wall intermediates, glycopeptides inhibit, apparently by steric hindrance, the formation of the backbone glycan chains (catalysed by peptidoglycan polymerase) from the simple wall subunits as they are extruded through the cytoplasmic membrane. The subsequent transpeptidation reaction that imparts rigidity to the cell wall is also thus inhibited. This unique mechanism of action, involving binding of the bulky inhibitor to the substrate outside the membrane so that the active sites of two enzymes cannot align themselves correctly, renders the acquisition of resistance to the glycopeptide antibiotics more difficult than that to the majority of the other antibiotic groups.

Vancomycin (1), the first glycopeptide antibiotic, was developed in the 1950s. It is effective at low concentrations against the majority of gram-positive bacteria, but toxicity problems encountered in the early years of its application precluded its widespread use in spite of its selective action against bacterial cell wall peptidoglycan. The introduction of β-lactamase-stable penicillins resulted in a temporary reduction in the clinical use of vancomycin, but the acquisition of alternative mechanisms of resistance to β-lactam antibiotics in particular and an improvement in the purity of the preparations has witnessed a resurgence in the use of glycopeptides, particularly against multiply resistant staphylococci that are intrinsically resistant to all β-lactam antibiotics (2). Vancomycin is the only glycopeptide antibiotic in clinical use throughout the world. Teicoplanin (3, 4) has recently been introduced in Italy and France and is under investigation in clinical trials in several European countries and in the USA.

Structure of Glycopeptides

Glycopeptide antibiotics are complex molecules of unique structure synthesized by a variety of species, including *Actinoplanes* and *Streptomyces*. The structure is based on a central, relatively conserved heptapeptide domain in which five of the seven amino acid residues are common to all glycopeptides (5, 6). Glycopeptides differ in the amino acids at positions 1 and 3 and in the substituents of the aromatic amino acid residues. In particular, some of the carbons of the aromatic residues carry chlorine, hydroxyl or methyl groups, and some of the hydroxyl groups are substituted with sugars or aminosugars, a number of which are found exclusively in a specific glycopeptide (7).

The presence of phenolic residues permits the formation of two- and three-ring structures in all the glycopeptides: such interactions result in a large group of molecules (at least 50 have been discovered) with very similar rigid three-dimensional structures. Those compounds that have rings 1 and 3 joined in addition to 2, 4 and 6, and 5 and 7, have been shown to adopt a bracelet-like configuration with a substantial cleft in the molecule into which the bacterial target site binds with exquisite precision (vide infra).

The basic structure containing the seven amino acid residues is termed the aglycone and is biologically active. The sugars and aminosugars found as substituents are located mainly on the outside of the molecule and do not markedly affect antibiotic activity in vitro; however, they are important in imparting different pharmacokinetic properties to the different glycopeptides. Little is known about the precise role of the sugars, but in ristocetin it is presumably these

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components that are ultimately responsible for causing blood platelet aggregation, which prevents the use of ristocetin as an antibiotic (8).

Some glycopeptides, including the teicoplanins (Figure 1) and the aridicins, have the amino group of an aminosugar substituted with a fatty acid chain containing nine to 11 carbon atoms (9). It is this substituent that confers greater hydrophobicity to the teicoplanin than to the vancomycin molecule. It remains to be seen whether molecules with different or additional hydrophobic substituents are able to penetrate the outer membrane of gram-negative bacteria and thus increase the spectrum of activity of this important group of antibiotics.

Mechanism of Action: Biochemical Studies

All biochemical studies of the mode of action of glycopeptide antibiotics indicate that these substances inhibit cell wall peptidoglycan synthesis. Treatment of intact bacteria with vancomycin at concentrations close to the MIC resulted in the accumulation of cytoplasmically located wall precursors (10, 11), suggesting that glycopeptides interfered with a late stage in the assembly of the peptidoglycan. The overall biosynthetic pathway of this polymer occurs in three stages: the first involves synthesis of the wall precursors in the cytoplasm; the second, formation of the wall subunit on a mobile lipid (undecaprenylphosphate) in the membrane followed by its transfer to the outer surface of the membrane; and in the third stage the growing glycan chain is attached to the new wall subunit by a transglycosylation reaction and is linked to the mature wall by transpeptidation (Figure 2). Vancomycin, and presumably other glycopeptides, cannot penetrate the cytoplasmic membrane (12), and thus the critical transglycosylation reaction is the first to be inhibited (13) (Figure 2). Inhibition of this reaction results in the accumulation of lipid intermediates in the biosynthetic pathway and of UDP-MurNAc-pentapeptide in the cytoplasm.

The actual target site is not the enzymic protein that catalyses the transglycosylase reaction. Rather, glycopeptides interact with a substrate of the enzyme and apparently shield it from the active site of the enzyme. The first evidence of this came from the observation that vancomycin formed a stoichiometric 1 : 1 complex with the peptidoglycan precursor UDP-MurNAc-pentapaptide (14). Systematic degradation of the nucleotide focussed attention on the carboxy terminus of the peptides as the region most important in complex formation. Testing of a large range of synthetic peptides established that acyl-D-ala-D-ala was the smallest molecule to form a complex with vancomycin and that the size and stereochemistry of the sidechains in the synthetic peptides determined the degree of freedom in the binding sites of vancomycin and ristocetin (15, 16). These studies were carried out several years before the structure common to the aglycones had been elucidated, and the results suggested that the three-dimensional binding sites of vancomycin and ristocetin were similar but not identical.

Glycopeptides bind with different affinities to groups of amino acids in the stereochemical configuration L-D-D. This explains why such large amounts of the antibiotics are bound to intact cells or isolated cell

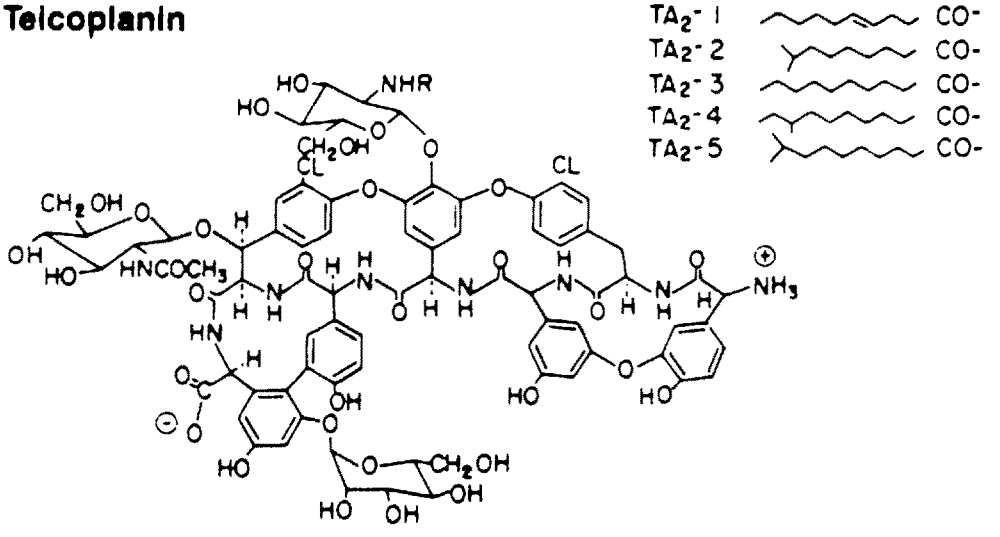

Figure 1: Structural representation of the teicoplanin complex. TA_2-1 to TA_2-5 are the components of the complex that are characterised by a fatty acid moiety at position R.

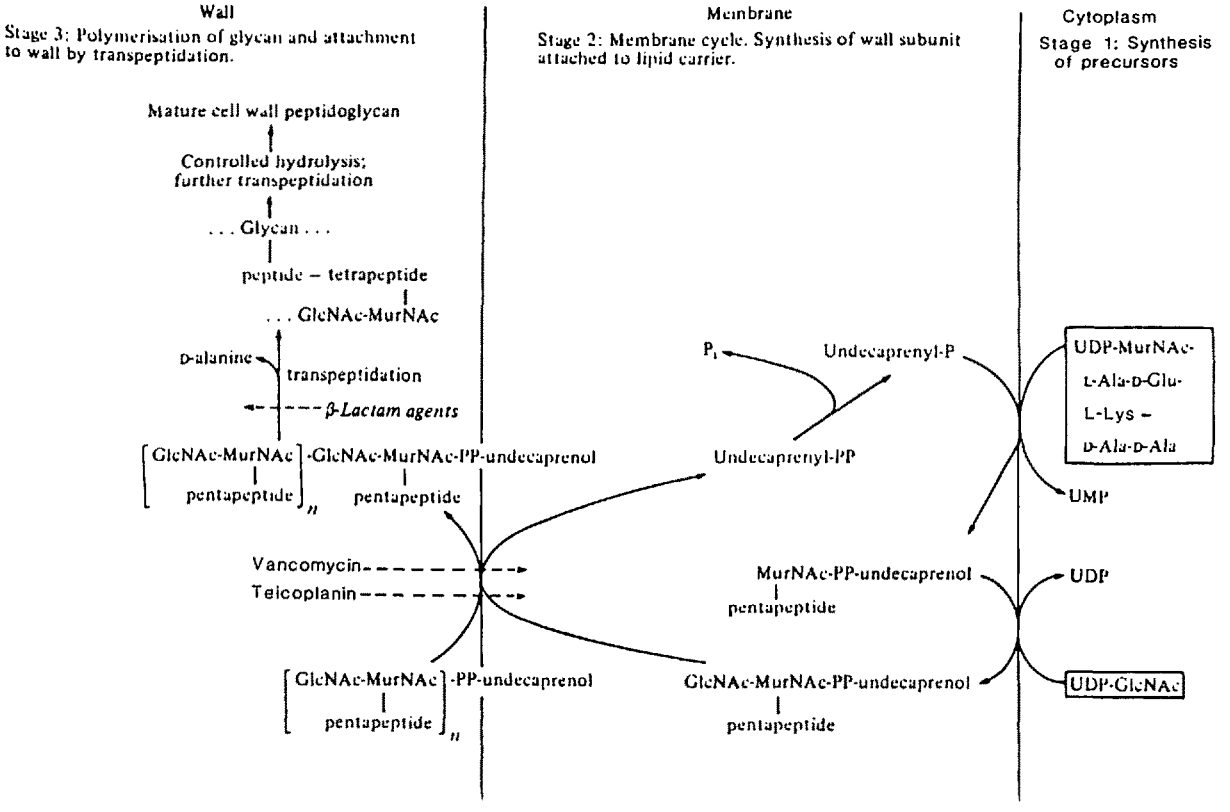

Figure 2: Generalised outline of the pathway of peptidoglycan biosynthesis. Additional amino acids present in the cross-bridge of some bacteria are normally added when the wall subunit is bound to undecaprenyl pyrophosphate. GlcNAc-N-acetylglucosamine; MurNAc-N-acetylmuramic acid.

walls (17, 18). The peptidoglycan component of many walls contains amino acid sequences that conform to the L-D-D principle in addition to *Laa*-D-ala-D-ala groups that are found particularly where new peptidoglycan subunits are being incorporated into the wall.

Consequently, when a glycopeptide interacts with a sensitive bacterium, it binds first, in large amounts, to the peptidoglycan component of the wall, which acts as an ion-exchange resin. Eventually, all the non-specific binding sites in the outer layers of the wall will be saturated, and free glycopeptide molecules will be available to bind to the important targets on the outside surface of the membrane, where new subunits are being incorporated. The actual target sites appear to be externally oriented wall precursors with peptides ending in *Laa*-D-ala-D-ala (Table 1). The most likely candidates are the lipid intermediate containing the disaccaride-pentapeptide wall subunit and the nascent peptidoglycan as it is extruded through the membrane prior to cross-linking (19).

Although vancomycin has been reported to be bactericidal, it is not necessarily the case that the bacteria are killed but that they are prevented from growing by the saturation of the available growth points of the peptidoglycan. The non-covalent nature of the binding of vancomycin to the important target sites is indicated by the ease with which the inhibition of either bacterial growth or peptidoglycan synthesis could be reversed. Such reversal has been accomplished by the addition to the growth or incubation medium of a suitable peptide that competed effectively with the natural wall peptides at the growth points for the available glycopeptide (20).

Table 1: Groups containing the sequence -*Laa*-D-*aa*-D-*aa* to which glycopeptide antibiotics bind.

1. UDP-MurNAc(pentapeptide) – inaccessible in intact cells
2. MurNAc(pentapeptide)-P-P-undecaprenol
3. GlcNAc-MurNAc(pentapeptide)-P-P-undecaprenol[a]
4. Nascent peptidoglycan[a]
5. Mature peptidoglycan (mostly non-specific)

[a]The most likely critical target sites in intact bacteria.

Mechanism of Action: Molecular Level

Detailed studies of the interaction of glycopeptides with the target site were dependent on a three-dimensional reconstruction of the binding sites. The three-dimensional structure of CDP-1, a crystalline compound derived from vancomycin, was published 12 years ago (21), but this structure has since been refined by the use of high field proton nuclear magnetic resonance spectroscopy and mass spectrometry (5). The chemical representation of a glycopeptide antibiotic, useful as it is for direct comparison of the many different structures, gives no indication of the spatial arrangement of the molecules. These arrangements have been elucidated either by the construction of space-filling molecular models (with due reference to all the spectroscopic data) in the case of vancomycin and ristocetin, or by computational methods and computer modelling (6), as for the aridicin aglycone (Figure 3).

Molecular modelling of glycopeptides provides an excellent representation of the compactness of these molecules and illustrates how the wall subunit ending in -D-Ala-D-Ala is held firmly by hydrogen bonding (5 such interactions can be formed; Figure 4) to the peptide backbone of the glycopeptide (7). The more recent computer-generated model of the aridicin aglycone illustrates clearly how the rigid antibiotic

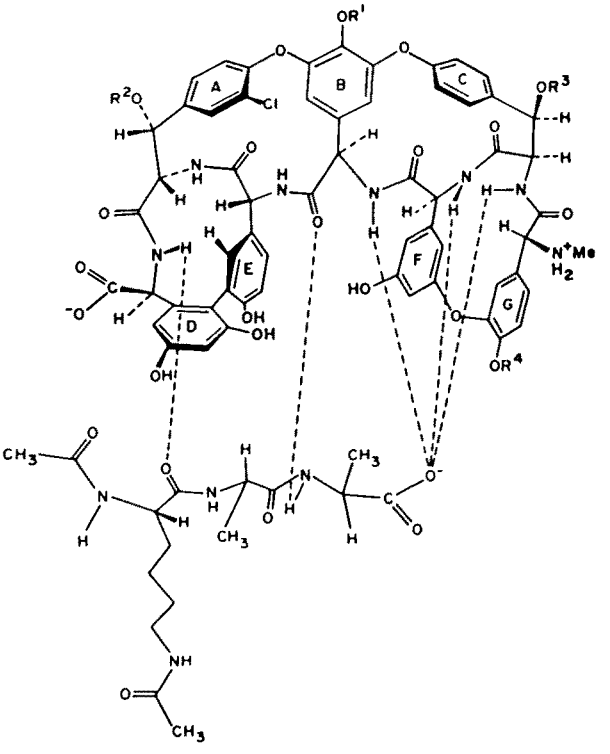

Figure 4: Diagrammatical representation of the interactions between a peptide ending in -D-alanyl-D-alanine and the aglycone of a glycopeptide.

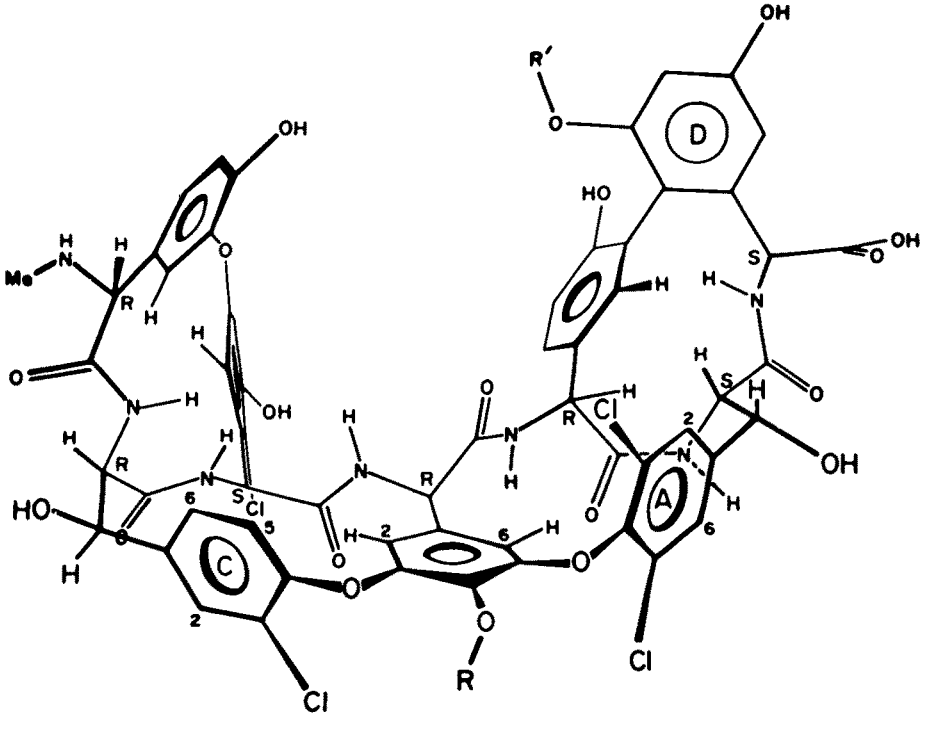

Figure 3: Three-dimensional model of the structure of the aridicin aglycone.

fits snugly around the -D-Ala-D-Ala portion of the peptidoglycan, which becomes buried in a cleft in the antibiotic molecule. The space-filling model of the glycopeptide-wall pentapeptide complex (6) indicates that the sugars in the wall subunit (GlcNAc-MurNAc) are not adjacent to the glycopeptide molecule (Figure 5); consequently, it is not immediately obvious why the transglycosylase enzyme that transfers a disaccharide-peptide from its lipid carrier to a nascent peptidoglycan chain is inhibited. Presumably, when the active site of the enzyme is in a position to cleave the muramyl-phosphate bond, part of the large protein molecule is in close proximity to the acyl-D-ala-D-ala part of the peptide. If this spatial position is occupied by a glycopeptide antibiotic in tight but non-covalent linkage to D-ala-D-ala, the correct alignment of enzyme and substrate cannot be achieved to effect the breakage/transfer reaction (Figure 6).

If a different or mutant transglycosylase enzyme could function in the presence of a glycopeptide antibiotic (perhaps resulting from a change in conformation so that the enzyme was not obstructed by the glycopeptide/D-ala-D-ala complex), the next reaction in peptidoglycan biosynthesis would also be blocked: a transpeptidation reaction in which newly synthesized nascent peptidoglycan is transferred to the existing mature peptidoglycan. The terminal D-alanine residue of the wall peptide is removed by an enzyme which itself becomes temporarily covalently attached to the preceding D-alanine residue of the peptide. This new enzyme-substrate complex reacts with another wall peptide to complete a cross-link that gives rigidity and strength to the peptidoglycan. A glycopeptide antibiotic would completely shield acyl-D-ala-D-ala residues from reaction with the transpeptidase enzyme (Figure 7). Therefore, even if some extension of the glycan chains could occur in the

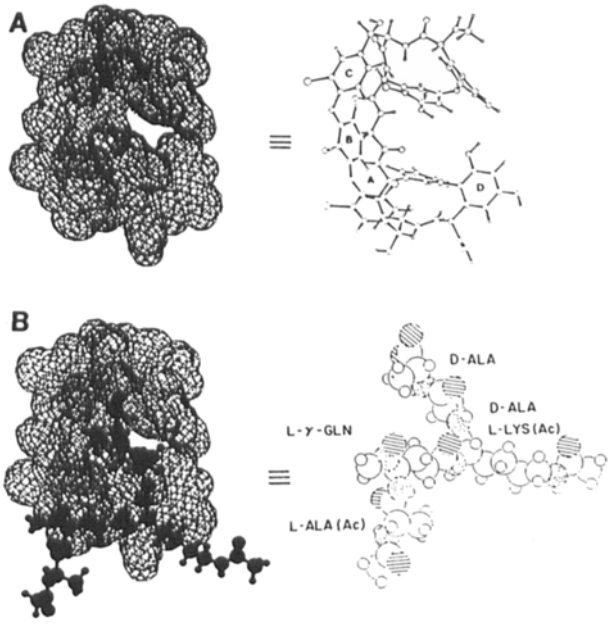

Figure 5: Computer-generated model showing space-filling representation of A) the aridicin aglycone and B) the complex formed between the aridicin aglycone and a wall pentapeptide (acetyl-L-Ala-D-Gln-L-Lys(Ac)-D-Ala-D-Ala). Structures were obtained using distance geometry with subsequent energy minimization.

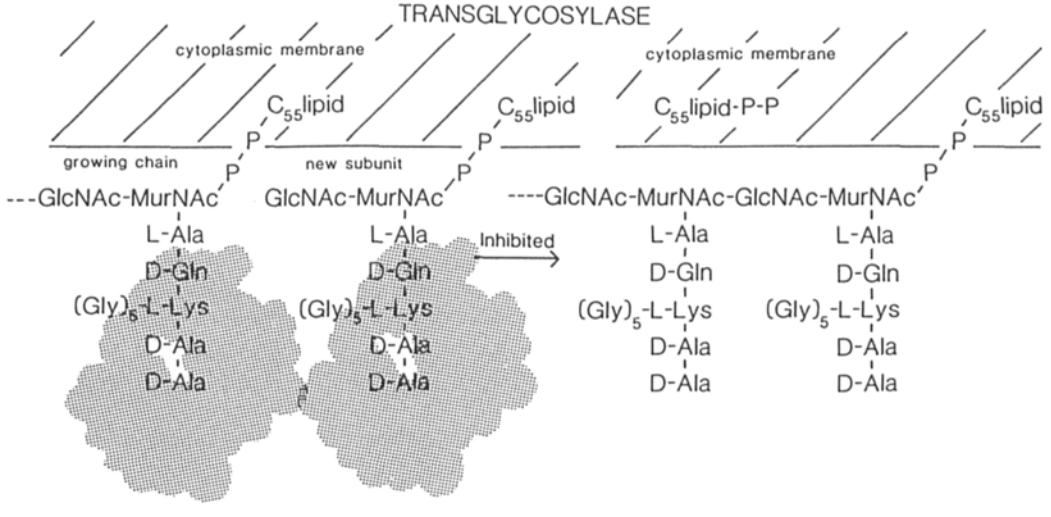

Figure 6: Diagrammatical representation of how glycopeptide molecules bound to -D-alanyl-D-alanine sequences in a new wall subunit and the growing nascent glycan chain may prevent, by steric hindrance, the transglycosylase reaction that links the nascent chain to the new wall subunit. The shaded area represents the approximate extent of the glycopeptide.

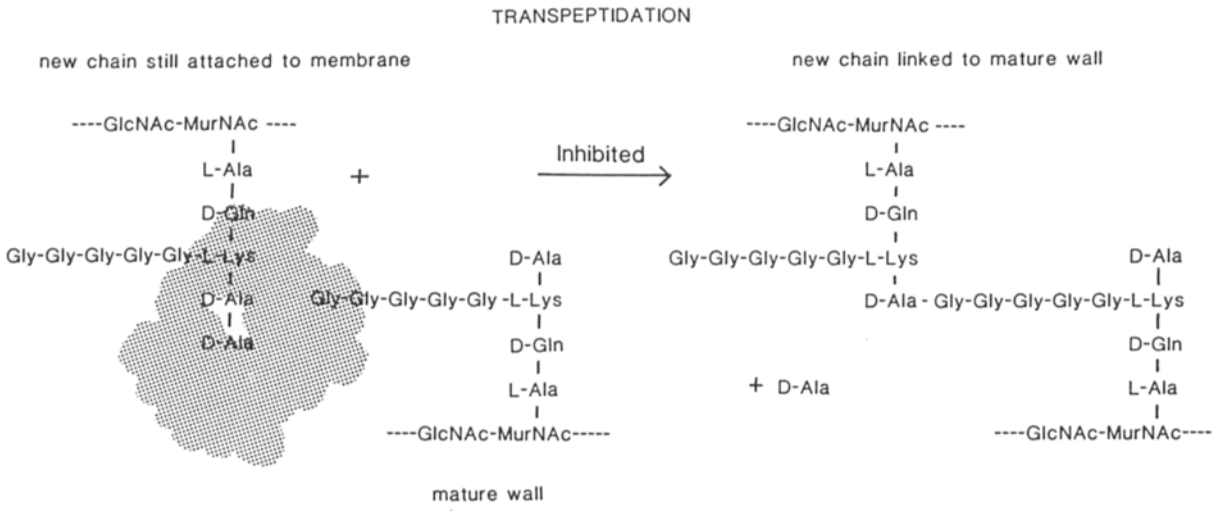

Figure 7: Diagrammatical representation of the inhibition of the cross-linking reaction (catalysed by a transpeptidase) in peptidoglycan synthesis by a glycopeptide. The shaded area represents the approximate extent of the molecule.

presence of glycopeptide antibiotics, there would be no possibility of these chains being transferred to the cell wall. There is effectively a double blockade present: two enzymes acting sequentially in the synthetic pathway are inhibited by glycopeptides.

As a result of the inhibition of these reactions, wall synthesis is frozen: the cell cannot increase in diameter, and DNA, RNA and protein synthesis is ultimately prevented. If autolytic enzymes continue to function in the absence of wall growth, cell death will follow, but this is unlikely to occur as rapidly as with β-lactam antibiotics.

Table 2: Possible mechanisms of resistance to glycopeptide antibiotics.

1. Inactivation of antibiotic
Proteolysis
Modification
2. Modification of target site
Change in pathway of peptidoglycan synthesis
3. Altered accessibility of target site
4. Sequestration of antibiotic

Resistance to Glycopeptides

The structure of glycopeptides, as well as their unique site and mechanism of action, is likely to ensure that any resistance mechanism acquired by a Gram-positive bacterium will be unusual compared to conventional mechanisms such as destruction or inactivation of an antibiotic or modification of the target site (22). Possible resistance mechanisms are listed in Table 2. Although the structure of glycopeptides is based on a linear heptapeptide, the presence of atypical residues, the compactness of the molecule and the location of the binding site in a cleft reduce the possibility of inactivation by cleavage of the peptide backbone. Modification of a variety of residues is possible, but such residues are unlikely to be involved in complex formation and any such alterations may not result in a significant decrease in affinity. It is significant that no reports of destruction or inactivation of glycopeptide antibiotics have yet appeared. It is also difficult to envisage development of resistance arising from a change in the target site because of the complexity of the peptidoglycan biosynthetic pathway: changes involving the complete refashioning of peptidoglycan synthesis could not be achieved rapidly, if at all.

It appears likely that recently reported resistance in coagulase-negative staphylococci (23) and in enterococci (24, 25, 26) results either from a change in the accessibility of the target site or from a decreased amount of antibiotic available at the target site due to sequestration of the substance in a modified wall structure or in the medium (Table 3). In some enterococcal strains resistance appears to be mediated through a 39-kilodalton protein (26) that may, in some undetermined manner, prevent access of glycopeptide molecules to the target site. However, not all strains are uniformly resistant to all glycopeptides; hence a simple barrier exclusion mechanism is unlikely.

Table 3: Possible mechanisms of sequestration of glycopeptide antibiotics.

	Alteration in metabolism	Mechanism of sequestration
1.	Decreased level of peptidoglycan transpeptidase activity	Wall is less cross-linked: more -D-Ala-D-Ala-groups to bind glycopeptides
2.	Thicker wall synthesized	More non-specific binding
3.	Increased rate of turnover of peptidoglycan in wall	More non-specific binding in medium
4.	Overproduction and excretion of wall intermediates into medium	Glycopeptides immobilised in medium

Glycopeptide-resistant staphylococci are considerably less resistant than the enterococcal strains that have been examined. Investigations with a strain of *Staphylococcus aureus* accustomed to growing in the presence of an elevated concentration of teicoplanin indicated that membrane preparations contained large amounts of a protein (M_r 33,000) that had been induced by growth in teicoplanin: the strain remained sensitive to vancomycin. In a parallel investigation large amounts of a 39-KDa membrane protein were synthesized constitutively by a teicoplanin-resistant, vancomycin-sensitive, clinical isolate of *Staphylococcus epidermitis* (unpublished observations). Since the walls of sensitive and resistant strains as well as the intact bacteria from which they were derived bound similar amounts of glycopeptide antibiotics, any mechanism relating to accessibility considerations is likely to operate very close to the membrane. Any proposed mechanism will also have to account for the resistance of the transpeptidation reaction in addition to the transglycosylation reaction. In staphylococci (27) and enterococci these two reactions are probably catalysed by different proteins, although both are membrane-bound.

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